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Ecological aspects of the resistance of plants
to environmental factors

Thesis presented by
Anthony Polwart, B.Sc.
for the degree of
Doctor of Philosophy in the Faculty of Science
in the
University of Glasgow

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PART I - INTRODUCTION

(a) General Introduction

The influence of the environment on the geographical or local distribution of plants has been studied and discussed for a very long time. As knowledge of floras grew, there developed a natural interest in explaining the distribution of species, and at the beginning of the nineteenth century Humboldt interpreted his observations on plant distribution throughout the world in terms of climate and climatic limitations. de Candolle (1855) expanded these ideas, emphasising the role of temperature in plant distribution. Further impetus was gained by Warming (1895, English transl. 1909) who placed much stress on water as a controlling factor. The basis of modern ideas on the complexity of the environment and the interaction of factors has come from Schimper (1898, English transl. 1903), and since then, intensive habitat investigations have been initiated. Schimper pointed out that "The oecology of plant-distribution will succeed in opening out new paths on condition only that it leans closely on experimental physiology" and it is from this point of view that these intensive studies have been carried out.

Schroeter (1926) summarised the results of European workers and outlined a fascinating picture of the life of alpine plants. Lundegårdh (1925, English transl. 1931) has gone into particular detail of the nature of environmental responses in plants and vegetation. He con-

sidered that "The aim of experimental ecology is the investigation of the plants reaction to a given 'constellation' of ecological factors, with a view to discovering how the plant behaves in its natural habitat."

This idea of a 'constellation' of ecological factors has been expanded and very well discussed by Billings (1952). He considered that, even although it has been customary for ecologists and plant physiologists to consider the effect of single factors on the plant, this is a rather artificial, though probably necessary, approach. He thinks that any study of plant growth and distribution in relation to the environment must consider each factor in relation to the others of the complex. Every plant species is distributed according to the tolerance ranges of its own ecotypes or biotypes. It is thought to grow where it does because the whole environment in time and space fits its genetic requirements, and time and the environment have allowed its seeds or propagules to reach that place. As the whole environment varies, then so will the communities resulting from such independent distribution vary gradually or sharply.

Billings (1952) saw the need for much more autecological work on tolerance ranges under field or simulated field conditions since individual plants of one or more species are the building blocks of vegetation, and although much information has been provided for cultivated plants few or no data on tolerance ranges exist for the great majority of wild plants.

Since Schimper (1898), much of the European work in this field has been of the autecological type with very intensive physiological studies in the field. This is even more so today with the work of Pisek, Tranquillini, Larcher and others referred to in other sections. There

have also been extensive studies of this nature in America by Parker, Kramer, Kozlowski and others, but it would seem that large scale analysis of vegetation and vegetation changes is still quite prevalent in environmental investigations.

An early attempt to correlate plant growth with the environment was by Sørensen (1941) on arctic plants in north-east Greenland. In a three year study he tried to elucidate the periodical life phenomena of the vegetation in an area whose climatic character is entirely dominated by the temperature factor. Although, like Billings (1952), he pointed out that "..... the vegetation does not react to the individual meteorological factors separately, but to the total effect of all climatic factors, the climate character.", he considered it justified in studying the single factor of temperature since vegetation rhythms between activity and rest appear to be closely correlated with the temperature climate. Another point stressed in his paper was seasonal periodicity, the temperature factor itself and its effects being viewed in relation to the seasons of the year. He also pointed out that if a plant species is to survive in the long run, it must in equal degree be adapted to the temperature climate prevalent in its habitat and with seasonal variation in the temperature climate. He considered that the unequal distribution of species between open, exposed areas and snow covered areas (more species on the latter) would seem to substantiate the importance of the thermic local climate to the plants.

Although many more studies on the environmental complex with respect to arctic and alpine plants have been carried out, it is usually found

that one or two factors have a predominant influence on the distribution of many of these plants. It is proposed to study in detail some of these factors as they influence certain physiological processes in these plants, and thus delimit the range of habitat of the plants.

Griggs (1946) attributed wind as influencing the altitude of the timberlines in North America. Wind was thought to be an important factor in the present upper limits of the Caledonian Forest in the Scottish Grampians (Pears, 1968), the level determined by temperature alone being significantly modified. Low temperature has been shown to influence vegetational patterns in the Rocky Mountains (Daubenmire, 1943), and Pinus pugnans appears to be limited by low temperatures because of injury to new growth or to reproductive processes (Zobel, 1969). Bliss (1956) has made useful correlations between the climate near the ground and arctic and alpine tundras and has found that higher temperatures near the ground in early summer had an important effect on the growth of certain arctic and alpine plants, though with less effect in the latter. High soil surface temperatures and deep non-rocky soil in the open, reducing available soil moisture, appear to maintain differences between the forest and open environments in Montana (Patten, 1963). Forest and Krummholz in Central Sierra Nevada are primarily restricted by abrasion caused by wind-driven snow and secondarily by high moisture stress and other factors (Klikoff, 1965). Michaelis (1934) has suggested that frost-drought, due to frozen soil and poor transpirational control by immature needles, is the controlling factor in determining the limit of the tree-line and timber-line in the Alps. Tranquillini (1967) has

confirmed this hypothesis and his findings are discussed in a later section. Troll (1956) has reviewed the influences of water on the distribution of plants.

Another influence affecting the growth of plants appears to be photoperiod (e.g. Vaartaja, 1959; Millener, 1962). Kaszkurewicz and Fogg (1967) found that variations in dates of the beginning and end of the growing seasons of Populus deltoides and Platanus occidentalis can be attributed to variations in air temperature and photoperiod.

Other environmental studies are reviewed in Billings (1957), Tranquillini (1964), Hiesey and Milner (1965), Billings and Mooney (1968) and Salisbury et al. (1968). An extensive coverage of climatic data and literature is given in the book on micro-meteorology by Geiger (1965).

Thus it can be seen that within the environmental complex, very often one or several factors can be selected as having the main influence on the distribution of the plants or on the individual plant response. The ability of established plants to resist environmental stresses at certain times of the year would also seem to be an important factor in their distribution. Three of the main factors in limiting distributions or affecting the growth of plants at a particular time of the year appear to be the temperature factor, the water factor and the light factor with particular reference to photoperiod.

The present study, as well as considering the distribution of the heath plants under investigation in light of the results, involves a more intensive investigation of physiological processes. It is the

aim of this investigation to consider some of the more important environmental factors (temperature, water and photoperiod) as they affect the physiology of a particular group of heath plants, and to consider the manner in which the plants have adapted to conditions of severe stress with particular reference to their variation in frost and drought resistance throughout the year.

(b) Choice of Material and Sites

Material

The genus Vaccinium has been selected for study because of the varied distributions of three species in the group. Vaccinium is a widespread, rather large and heterogeneous, genus of more or less shrubby perennials, having erect aerial stems and short-petioled, alternate leaves that are simple and usually deciduous. In Britain, the genus is often a dominant undershrub in forests and woods and a dominant or co-dominant on heaths or high altitude moors.

Vaccinium myrtillus L. is a deciduous, rhizomatous shrub which is widely distributed throughout Britain, though less prevalent in the east, being absent in East Anglia and the South-East. There is no evidence which indicates that the distribution is due to climatic factors as it ranges from sea-level (very oceanic) to 4,000 ft. (1,216 m) (high alpine). It is very common in the moors and woodlands of the Scottish Highlands and shows its maximum vegetative and reproductive performance in open pinewoods (Ritchie, 1956). V. myrtillus is markedly tolerant of shade and is thus often found dominant in pine and oak-woods. In the latter

habitats it reaches a height of 60-90 cm. but on montane summits and exposed cliffs it is seldom taller than 5 cm. However there is considerable variation within a clone in the latter habitat (Bright, 1928), shoots in sheltered niches being 3-5 cm. taller than those in fully exposed ones.

Vaccinium vitis-idaea L. is a rhizomatous, evergreen shrub with a more restricted distribution than V. myrtillus, being of a continental-alpine type. V. vitis-idaea extends from about 500 ft. (152 m) to 3,500 ft. (1,067 m) in mountainous areas only, and is entirely absent from the more oceanic areas, e.g. S.W. Ireland. The complete absence of this species from the heaths of south England, which appear to be edaphically suitable, would appear to be explicable in terms of climatic factors. It has been suggested (Ritchie, 1955) that the maximum summer temperature is the factor which shows the most significant difference between the southern limit of the plant and those southern heaths from which it is absent. Ritchie (1955) also suggests that altitudinal limits may be determined by snow cover and exposure to frost since there is evidence for this in the mountains of Scandinavia.

V. vitis-idaea attains its maximum reproductive performance in pinewoods, and of unshaded habitats is common on rocky ledges and screes. It is also an important species in "Vaccinium edge" communities of the Pennines. In forests and woods it forms large patches, the shoots reaching 20-30 cm. in length, and similarly in lowland heaths and drained bogs. As with V. myrtillus, much variation in single clones on montane heaths is seen with V. vitis-idaea.

Vaccinium uliginosum L. is a bushy plant 2-60 cm. in height, having a creeping rhizome and strongish woody branches. Apart from a few localities in the Pennines and the Southern Uplands of Scotland, this species appears to be restricted to the Central and N.W. Highlands of Scotland. At its southernmost limit, it usually has a lower altitudinal limit of about 1,800 ft. (550 m), though in the north of Scotland and in Orkney and Shetland it grows down to 900 ft. (274 m).

In the S. Central Highlands, V. uliginosum appears restricted to rock ledges which are fairly sheltered and moist. Further north it occurs in peat bogs and other similar habitats which are not too dry (e.g. Burges, 1951).

All three species are very common heath, woodland and montane plants on the Continent, and in Britain V. myrtillus and V. vitis-idaea would appear to be at their western limit. In Britain, V. uliginosum, which is a very important constituent of northern heaths, having a completely circumpolar distribution, is at its southern and western limit, (Polunin, 1959).

On the Continent, physiological studies have been carried out on all three Vaccinium species from an ecological point of view (e.g. Pisek and Cartellieri, 1933; Hygen, 1951, 1953a, b), and in the present study it is hoped to supplement and complement the researches of these Continental workers.

Sample sites - Scotland - see summary Table 2.

Montane habitats

After investigation, a suitable area for sampling all three



Plate 1. Ben Lui, Argyll, viewed from the east.

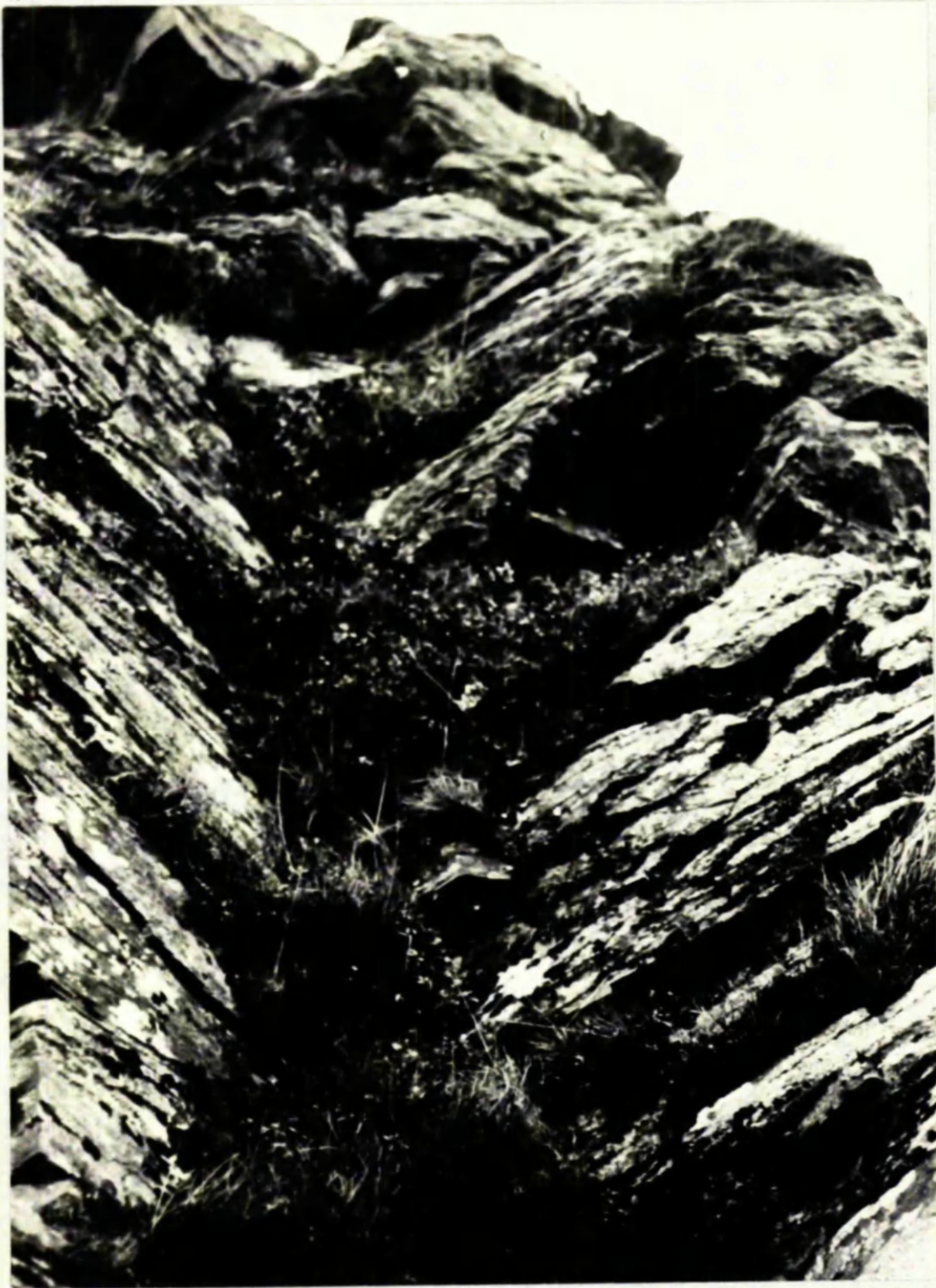


Plate 2. Rock ledge habitat, Ben Lui.



Plate 3. Turf habitat, Ben Lui; knoll in foreground and ridge to extreme right of photograph.

Vaccinium species was found on the east side of Ben Lui which lies on the boundary between Argyllshire and Perthshire (Plate 1). On Ben Lui, as well as on all other montane areas investigated, it is evident that two types of habitat exist with respect to Vaccinium myrtillus and V. vitis-idaea. In the first habitat, rock ledges (Plate 2), both species grow quite large with heights of up to 30 cm. for V. myrtillus and up to 20 cm. for V. vitis-idaea. The leaves of these forms are also large, those of V. vitis-idaea being up to 25 mm. in length. In the turf habitat (Plate 3), neither species grows greater than 10 cm. in height, though usually about 5 cm, and their leaves are small, less than 10 mm.

Investigations have been carried out on the V. vitis-idaea from these two habitats and that of Loch Katrine, to determine if any significant differences in morphological characters could be detected. Characters measured were leaf area, number of stomata per unit area, stomatal length and leaf area ratio (LAR). [To avoid differences between leaves on the same shoot, the fourth leaf from the apex of the shoot has been examined for twenty shoots.]

Very highly significant differences are seen with respect to leaf area and stomata per unit area. The other two parameters are also significantly different. The importance of these differences to the physiology of the plants is discussed in the section on drought resistance.

Direct measurement of stomata has not been possible with this tissue, so a method using surface replicates (cf. Sampson, 1961) has been used.

Leaf areas have been determined by the cut card method, and results are given in Table 1.

Table 1. Morphological measurements of *V. vitis-idaea*
from three habitats

Character	Loch Katrine	Ben Lui, ledge	Ben Lui, turf	
Leaf area (mm^2)	177.88	112.73	50.89	***
Stomata (no./ mm^2)	279.69	334.78	457.54	***
Stomatal length (nm)	26.342	26.093	24.448	**
LAR ($\text{mm}^2, \text{mg}^{-1}$)	9.16	7.31	9.52	*

*** significant difference, $p < 0.001$

** significant difference, $p < 0.01$

* significant difference, $p < 0.05$

On Ben Lui, *V. uliginosum* grows only on rock ledges which are usually quite moist and often have water running over them. It grows in a well-branched, bushy habit up to a height of 30 cm.

The ledges have only one aspect and are often sheltered from the wind and at some times shaded from direct sunshine. On a clear day in winter, it has been estimated that many rock ledges (e.g. Plate 3) receive no more than 1.1/2 hours of direct sunlight.

On the other hand, the turf habitat is often very exposed, and tends to dry out very quickly unless the soil underneath is saturated. This habitat is much more open than the rock ledges and receives more direct sunlight. The turf habitat would appear to be subject to more



Plate 4. Open woodland, Loch Katrine, with
typical mixed clumps of
Vaccinium myrtillus and V. vitis-idaea.

severe environmental stress than the rock ledge habitat.

On the rock ledges, pure or mixed stands of the three Vaccinia are very often observed. Other species occurring are Salix herbacea, Empetrum nigrum, and Festuca ovina, whilst on the more basic ledges a rich bryophyte flora is seen along with calcicolous species such as Saxifraga oppositifolia, S. stellaris, Silene acaulis and Sedum rosea.

The more exposed turf habitat supports a very sparse vegetation in comparison with the ledges, the most common species associated with V. myrtillus and V. vitis-idaea being Festuca ovina and the bryophyte Racomitrium lanuginosum.

Samples of V. myrtillus and V. vitis-idaea have been taken from a rock ledge at 2,150 ft. (654 m) with a S.E. aspect (Plate 2), and V. uliginosum has been taken from a ledge below this at 2,000 ft. (608 m) with the same aspect. Turf samples of V. myrtillus and V. vitis-idaea have been taken from an exposed knoll (Plate 3) at 2,150 ft. (654 m).

Woodland habitats

Two woodland sites have been chosen, one containing both V. myrtillus and V. vitis-idaea, the other only V. myrtillus.

Quite open woodland, consisting mainly of oak (Quercus species) and birch (Betula species) is found at Loch Katrine, Perthshire, (Plate 4). The ground flora consists of large pure patches of mixed V. myrtillus and V. vitis-idaea, though in the more open areas Calluna vulgaris is dominant. The size of the plants is larger than that of the rock ledge types, with heights of both V. myrtillus and V. vitis-idaea up to 60 cm. Leaves of V. vitis-idaea are up to 30 mm. long. Vegetative growth in



Plate 5. Shaded woodland, Garelochhead, with large
pure stand of Vaccinium myrtillus in the centre

1925



Plate 6. Open heath, Milngavie Moor, with
Vaccinium myrtillus and Pteridium
aquilinum (top right).

this habitat thus appears to be quite extensive. The habitat is shaded and quite dry, especially in the summer. A site was chosen on a steep slope (30°) with a West aspect at 450 ft. (137 m) above mean sea level (M.S.L.), 50 ft. (15 m) above the level of the loch.

The second woodland site is situated at Garelochhead, Dunbartonshire. The V. myrtillus is situated on the side of a gully (Plate 5) through which runs a stream. The gully is shaded quite considerably, especially in summer, mainly by oak (Quercus species), but also occurring are hazel (Corylus avellana), ash (Fraxinus excelsior) and birch (Betula species). Pure stands of V. myrtillus are seen, but in places near the edge of the gully it is mixed with Erica cinerea and Calluna vulgaris. Vegetative growth of V. myrtillus is extensive, with the length of shoots being up to 60 cm. The site is more shaded than Loch Katrine and is dry. It stands 200 ft. (61 m) above M.S.L. on the edge of a gully running in a N.W.-S.E. direction. The site does receive some direct sunshine, even in summer when the trees are in leaf.

Heath habitat

An open heath site on Milngavie Moor, Dunbartonshire has been chosen. Pure stands of V. myrtillus are seen (Plate 6), though bracken (Pteridium aquilinum) invades many of them in summer. The V. myrtillus in this habitat is intermediate in size, growing to a height of about 15 cm. The site, at 350 ft. (106 m) above M.S.L., is level, very open and exposed and tends to be dry. This site was destroyed by fire in April, 1969, and another site, similar to the first, was selected nearby.

Sample sites - England - see summary Table 2a.

(Material has been kindly collected by Mr. Paul Tregenza, Malham Tarn Field Centre, Mr. Colin Reynolds, Preston Montford Field Centre, Mr. Malcolm Litterick, The Leonard Wills Field Centre, and Mr. John Griffith, Slapton Ley Field Centre).

"Vaccinium edge" community

V. myrtillus and V. vitis-idaea have been collected from a "Vaccinium edge" community at Malham Tarn, Yorkshire. The plants in this habitat are quite large, with heights of up to 20 cm. like the lodge types, Ben Lui. The site is on the edge of a raised bog occupying part of a lake basin. The boundary between the moss and the tarn is an east-facing, 10 ft. high, eroded peat cliff with "Vaccinium edge" vegetation on top. The site is open, and situated 1,250 ft. (380 m) above M.S.L.

Mountain heath habitat

V. myrtillus and V. vitis-idaea have been collected from a mountain heath community on the Stiperstones, Shropshire. The plants are similar in size to those of the "Vaccinium edge" community at Malham Tarn. Associated with the Vaccinia are Calluna vulgaris, Ulex gallii, Melampyrum pratense and occasionally Empetrum nigrum. The site is open and exposed on a 5° slope facing west at a height of 1,400 ft. (425 m) above M.S.L.

Southern heaths

Vaccinium myrtillus has been collected from Dartmoor, Devonshire. The leaves of the plants are similar in size to those on Milngavie Moor, Dunbartonshire, but their habit is unknown. With it are associated Erica cinerea, Calluna vulgaris, Ulex europaeus and Pteridium aquilinum. The site is open on a 5° slope facing south-east at a height of 950 ft. (290 m) above M.S.L.

Table 2a. Sites from which material was collected for study

Locality	National Grid Reference	Height above Mean Sea-level Feet Metres	Exposure	Shade	Species Collected
Ben Lui (a) ledge 1) 2)	27/276265 27/277266	2150 (654) 2000 (608)	Sheltered compared to turf	Shaded compared to turf	<u>Vm</u> and <u>Vvi</u> <u>Vu</u>
(b) turf	27/273269	2150 (654)	Very exposed	Open	<u>Vm</u> and <u>Vvi</u>
Loch Katrine	27/496075	450 (137)	Sheltered	Shaded	<u>Vm</u> and <u>Vvi</u>
Garelochhead	26/232904	200 (61)	Sheltered	Shaded	<u>Vm</u>
Milngavie 1) to April, 1969 Moor 2) from April, 1969	26/553763 26/549762	350 (106)	Exposed	Open	<u>Vm</u>
Malham Tarn	34/889669	1250 (380)	Exposed	Open	<u>Vm</u> and <u>Vvi</u>
Stiperstones	32/362993	1400 (425)	Exposed	Open	<u>Vm</u> and <u>Vvi</u>
Dartmoor	20/681638	950 (290)	Exposed	Open	<u>Vm</u>
Exmoor	21/866415	1550 (471)	Very exposed	Open	<u>Vm</u>

Vm = Vaccinium myrtillus; Vvi = Vaccinium vitis-idaea; Vu = Vaccinium uliginosum

V. myrtillus has also been collected from Exmoor in Somersetshire.

The plants are similar in size to the Dartmoor material but again their habit is unknown. The site is on the east side of Exmoor and the associated flora is mainly Calluna vulgaris. It is very exposed and lies 1,550 ft. (395 m) above M.S.L.

All sites have been summarised in Table 2a with National Grid References given for their location.

(c) Environmental measurements

Ben Lui

A convenient, ungrazed ledge was found at 2,250 ft. (686 m) which supports all three Vaccinium species, and instruments for measuring temperature were set up (Plate 7).

A Grant automatic temperature recorder with nine thermocouples has been set up on the ledge (Plate 8) and readings are recorded every hour. The positions of the thermocouples are as follows:

- (1) 40 cm. above ground level, shielded.
- (2) Rhizome level, 3-5 cm. below ground level.
- (3) 30 cm. above ground level, shielded.
- (4) Ground level, unshielded.
- (5) 20 cm. above ground level, shielded.
- (6) Taped to stem of Vaccinium myrtillus, unshielded.
- (7) 10 cm. above ground level, shielded.
- (8) Taped to stem of Vaccinium uliginosum, unshielded.
- (9) Taped to stem of Vaccinium vitis-idaea, unshielded.



Plate 7. Ledge site, Ben Lui, supporting all three Vaccinium species
on which temperature recorder was set up.



Plate 8. Close up of ledge site, Ben Lui, showing shielded thermocouples, leads and temperature recorder box inside a heavy polythene bag.

The shield consists of a small fruit juice can with the ends cut out, suspended horizontally inside a soup can also with the ends cut out. The outer can was subsequently covered in aluminium foil. This arrangement gives an air jacket to stabilise temperature fluctuations due to direct radiation. The thermocouple is suspended horizontally inside the small can. Four of these shields are fixed horizontally to a vertical cane at several heights above the ground to determine variations in micro-environment close to the ground.

Unfortunately, due to technical faults, measurements have been irregular over the sampling period, and it has been necessary to use mean temperatures from the nearby weather station at Sloy, and adjust these temperatures for altitudinal differences between the Ben Lui site and Sloy. Sixty-six mean daily temperatures at Ben Lui have been compared with the equivalent sixty-six mean daily temperatures at Sloy.

The regression equation of Ben Lui on Sloy, $\hat{Y} = 1.1867X - 6.287$, $r = 0.9126^{***}$ ($p < 0.001$), indicates a curvilinear relationship and it has been considered that, since many of the values used in this study are in the middle of the range, a practical conversion factor would be of more use. For this purpose, the arithmetic mean of the differences between individual pairs of values has been calculated and comes to 3.89°C . This means that mean temperatures on Ben Lui at 2,250 ft. (686 m) are approximately 4°C lower than mean temperatures at Sloy, and this correction value has been used in calculating mean weekly temperatures on Ben Lui. 4°C between the two localities is equivalent to a temperature drop of 1°C for every 550 ft. (167 m) increase in altitude.

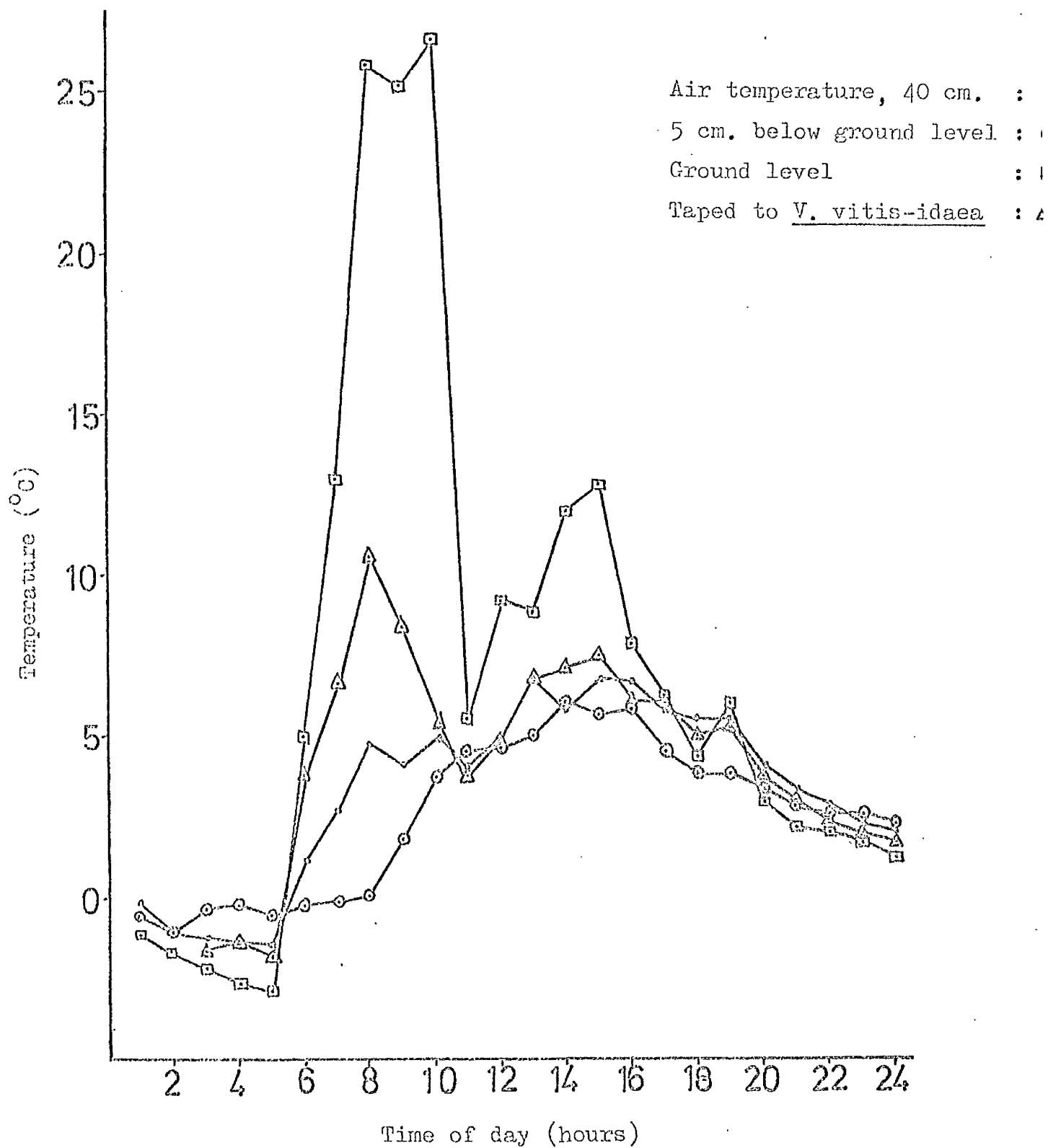


Figure 1. Diurnal variation in temperature, Ben Lui,
20th May, 1968.

Little variation between the temperature recorded by individual shielded thermocouples has been observed, and the mean daily temperatures used in the above regression have been calculated by taking the average of the daily means for each of the four thermocouples. The recordings of the other thermocouples are rather irregular for use in a seasonal study but it is interesting to note that a ground-surface temperature of 44.5°C was recorded in June, 1968, and the unshielded thermocouples taped to the plants have given readings of up to 35°C in that same month.

The diurnal variation of temperatures recorded by four thermocouples on a sunny day in late Spring (20th May) is shown in Figure 1. It can be seen that the two unshielded thermocouples, one at ground level and one taped to shoot of V. vitis-idaea, show much larger variations than the shielded air temperature and subterranean thermocouples. It is probable that the temperature of the plants is several degrees higher than ambient temperature when subjected to direct sunlight, and this may facilitate greater metabolism when the ambient air temperature is low. The mean air temperature of 20th May was 2.5°C , with the temperature rising above 5°C for only 7 hours. The unshielded thermocouple taped to V. vitis-idaea gave a mean of 4.8°C and exceeded 5°C for 10 hours.

Even at this time of year (late Spring), the sun impinges on the ledge for no more than 3 hours. The very sharp drop in ground level temperature between 10.00 and 11.00 hours is due to the disappearance of direct sunlight on the ledge. The temperature at rhizome level

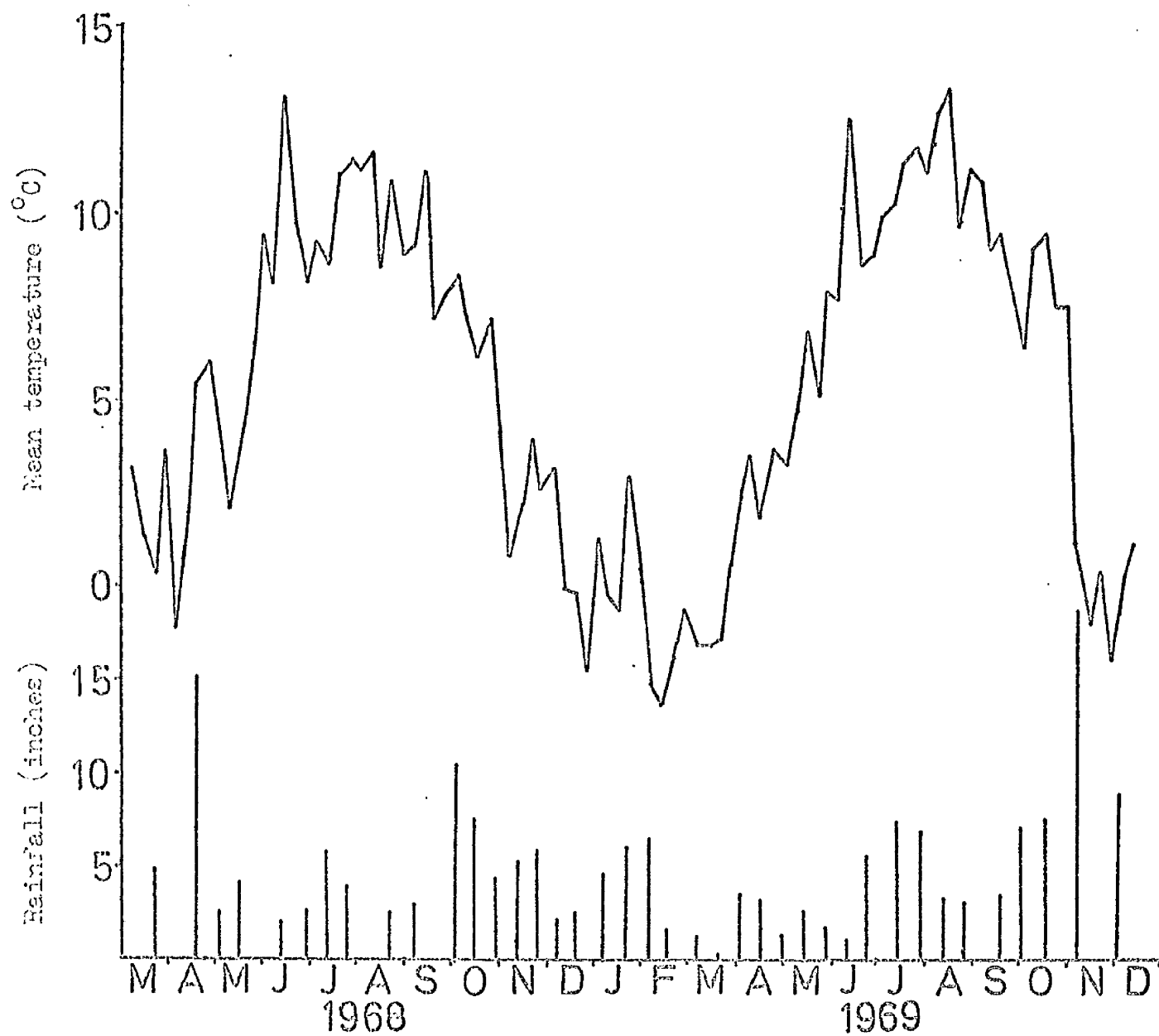


Figure 2. Seasonal course of precipitation and mean weekly temperature, Ben Lui.

shows a lag in response at the beginning of the day with zero or sub-zero temperatures being maintained for 8 hours, during which the unshielded thermocouples are approaching their maximum temperatures. The probable effect of this on the physiology of the plants is discussed in the section on drought resistance.

On days with little or no sunshine, the temperature of the unshielded thermocouples shows little or no variation compared to that of the shielded ones.

Using the adjustment of 4°C , mean weekly temperatures have been calculated for the Ben Lui site and are presented in Figure 2. A rain gauge has also been set up near the site and the rainfall measured regularly. These measurements have also been included in Figure 2, each line representing the rainfall for the period since the previous measurement.

All other sites

At no other site have measurements been taken in situ. The mean weekly temperature and rainfall data for Loch Katrine, Garelochhead and Milngavie Moor have been extracted from the records of the stations at Callander, Helensburgh and Springburn Park, Glasgow respectively (see summary, Table 4). There is a weather station very close to the site at Malham Tarn and data have been extracted from their records. No mean weekly temperatures have been calculated for Stiperstones, Dartmoor and Exmoor since records are not easily obtainable, but mean monthly figures have been taken from nearby stations as recorded in the 1969 Monthly Weather Report of the Meteorological Office. The weather

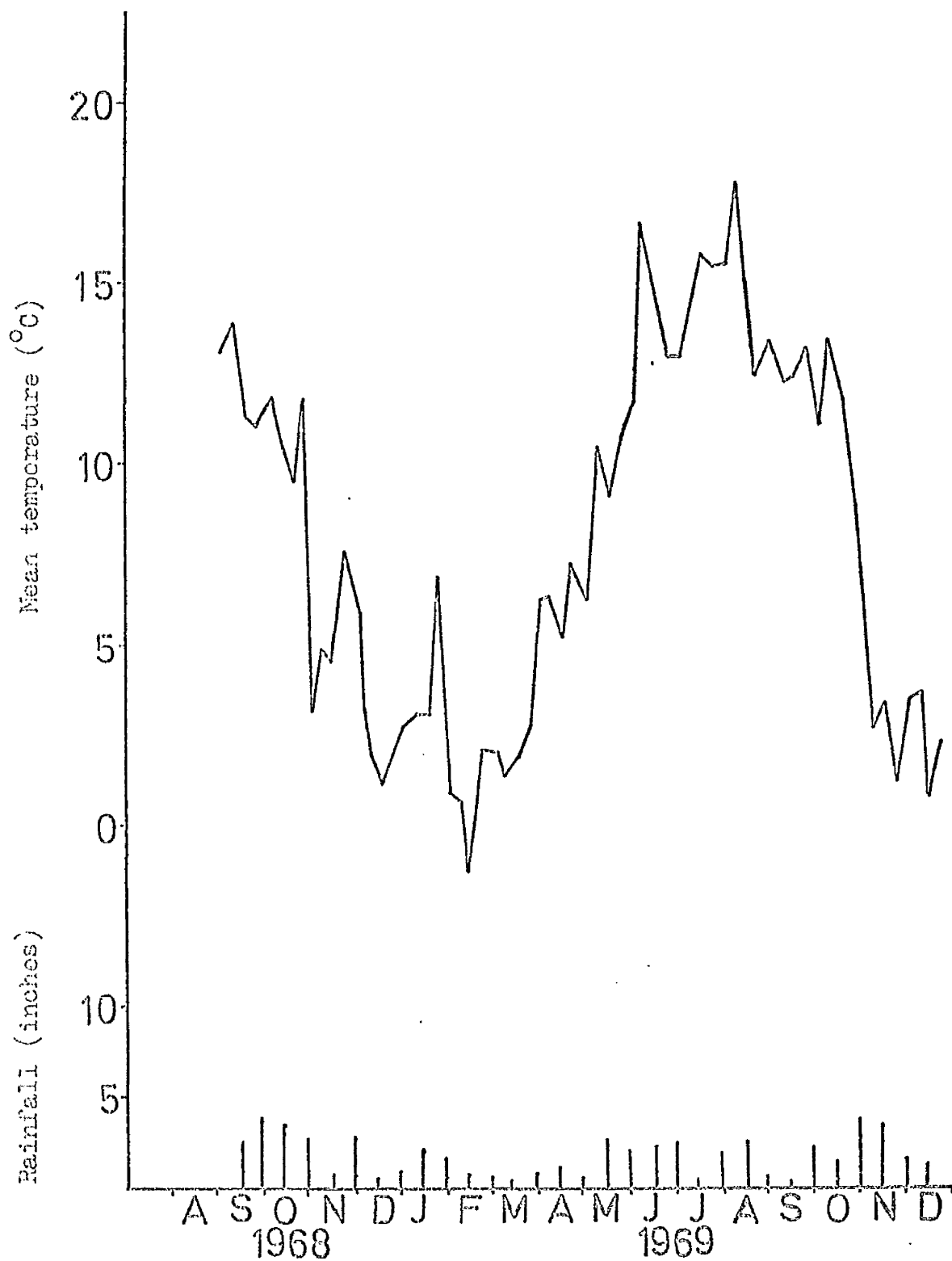


Figure 3. Seasonal course of precipitation and mean weekly temperature, Springburn Park.

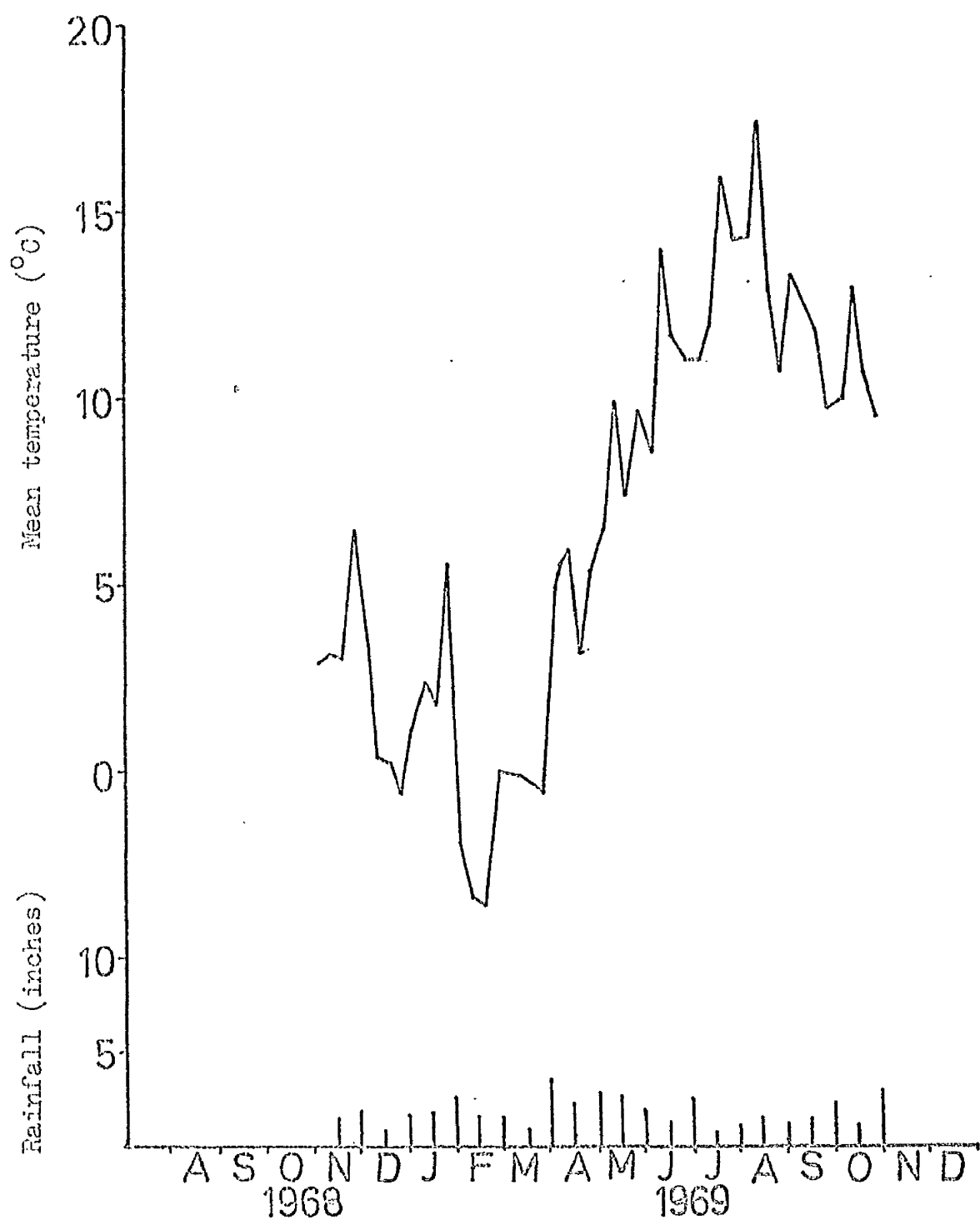


Figure 4. Seasonal course of precipitation and mean weekly temperatures, Malham Tarn.

Table 3. Monthly rainfall (inches). 1968-69

Locality	N	D	J	F	M	A	M	J	J	A	S	O	Total
Ben Lui	10.37	6.46	15.22	3.10	4.20	4.86	4.52	6.77	14.33	6.36	3.23	14.86	94.28
Callander	5.56	2.84	5.95	1.94	0.94	2.31	4.60	4.83	2.77	3.73	4.25	4.47	44.19
Helensburgh	3.74	2.08	4.52	1.48	0.96	2.19	4.56	3.38	2.84	3.89	3.75	4.00	37.39
Springburn	3.38	1.43	3.66	1.34	0.73	1.67	4.65	2.83	2.59	3.50	2.84	2.62	31.24
Malham Tarn	3.17	2.41	4.46	1.85	4.68	4.87	4.45	3.70	1.73	2.63	3.64	2.97	40.56

stations selected to represent the latter three sites are Shrewsbury, Tavistock and Hawkhill respectively. Temperature means have been adjusted for altitude where necessary, using a standard adjustment of 1°C per 500 ft. (150 m) (cf. Oliver, 1964). In order to compare these three sites with other localities, mean monthly temperatures have also been calculated for Malham Tarn and Milngavie Moor. All five sets of data are presented in Table 2.

Table 2. Mean monthly temperatures ($^{\circ}\text{C}$) at five localities, 1969

Locality	J	F	M	A	M	J	J	A	S	O
Milngavie Moor	4.0	0.5	2.4	6.4	9.3	13.5	14.8	15.5	12.3	11.9
Malham Tarn	2.7	-2.2	0.2	4.8	8.6	11.4	13.3	15.0	10.9	10.6
Stiperstones	3.4	-1.4	1.1	5.4	9.2	11.8	15.1	14.2	11.4	-
Exmoor	3.8	-0.1	2.1	5.5	8.9	11.2	13.6	13.2	11.4	10.8
Dartmoor	5.4	1.3	4.4	7.3	9.2	12.7	14.9	14.4	13.2	12.1

Graphs of mean weekly temperatures for Springburn Park and Malham Tarn are presented in Figures 3 and 4, along with rainfall measurements. The mean weekly temperatures at Helonsburgh and Callander differ only slightly from those at Springburn Park and have not been presented. Rainfall does differ, however, and monthly figures from November, 1968 to October, 1969 for five sites are presented in Table 3.

It can be seen that the montane site of Ben Lui is by far the wettest area, with Springburn Park, Glasgow the driest area.

A summary of weather stations with National Grid References is given in Table 4.

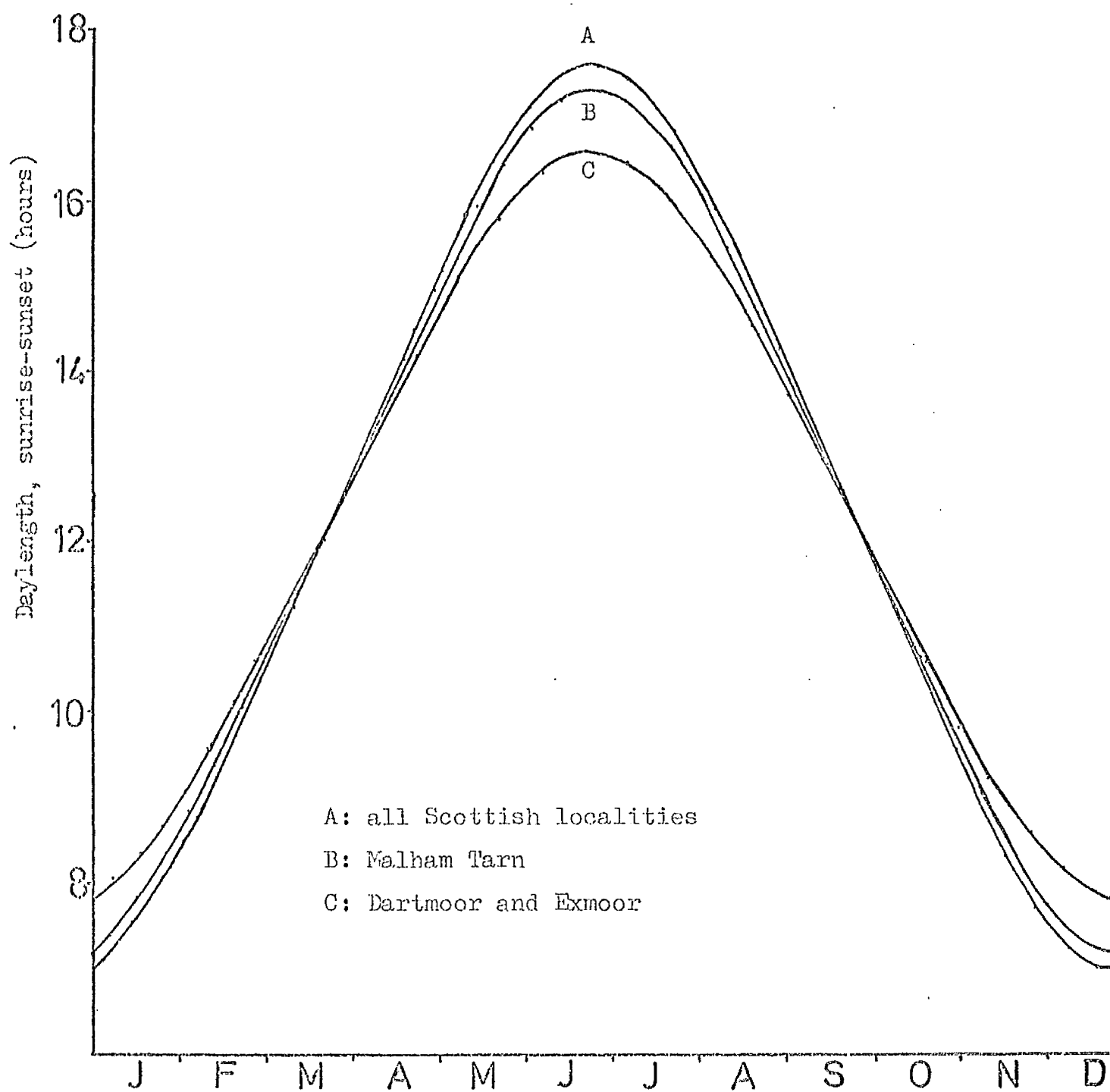


Figure 5. Seasonal variation in daylength, sunrise to sunset.

Table 4. Location of Weather Stations

Locality	National Grid Reference	Height above sea-level		Temperature adjustment
		Feet	Metres	
Ben Lui				
1) Temperature Recorder	27/272269	2250	(684)	-
2) Rain gauge	27/274268	2100	(636)	-
Sloy	27/324099	40	(12)	-4°C for Ben Lui
Callander	27/634080	350	(107)	None
Helensburgh	26/303836	293	(89)	None
Springburn Park	26/608686	351	(107)	None
Malham Tarn	34/894673	1300	(395)	None
Shrewsbury	33/517136	175	(53)	-2°C for Stiperstones
Hawkridge	21/877327	1000	(304)	-1°C for Exmoor
Tavistock	20/482748	500	(152)	-1°C for Dartmoor

Daylength data has been extracted from tables, and seasonal variation in photoperiod, sunrise to sunset, is presented for three localities in Figure 5. It can be seen that Scottish localities have longer daylengths in summer and shorter daylengths in winter compared to those in the south of England. Daylengths at Malham Tarn are intermediate in length in summer and winter.

PART II - FIELD STUDIES

(a) Frost Resistance1. Introduction

The ability of plants to withstand extreme variations in environmental factors has been described either as resistance or hardiness to any particular factor. Confusion between these two terms has arisen, and here it is intended to use resistance in the broad sense of tolerating an unfavourable external environment. Hardiness will be used with respect to a more specific tolerance of an environmental factor e.g. frost hardiness means the ability of the plant to survive being frosted.

Before considering the broader ecological aspects of frost resistance, some basic principles will be described. The more historical literature on this subject has already been considered by Levitt (1956).

Causes of frost damage

Injury of plants due to extremes of temperature, either high or low, is due to the physiological balance of the plant being upset and consequently irreversible changes taking place in individual cells. With respect to low sub-zero temperatures, several stages can be delimited within the continuous process of freezing and thawing (Mazur, 1969).

1. As the temperature drops below 0°C , both cells and the medium supercool but ice soon forms in the medium. Above -10°C the

cell membrane keeps ice from seeding the cell interior.

2. As more external solution is converted to ice, the concentration of all extracellular solutes rises and the aqueous vapour pressure falls. Water thus flows out of the cells and freezes extracellularly. The resulting dehydration concentrates the intracellular solutes and decreases distances between the larger molecules. The extent of cell dehydration to maintain equilibrium depends on the permeability of the cell membrane or the rate of cooling. Intracellular freezing will only occur if permeability is not sufficient or if cooling is too fast.
3. There may be large pH changes if solubility is exceeded and solutes are precipitated.
4. All free water is converted to ice and all solutes are precipitated below the eutectic point. Above this temperature, a cell is immersed in, and also contains, a highly concentrated solution whose physical properties differ considerably from the normal.
5. Small ice crystals have the tendency to convert to large spherical crystals.
6. During warming a reversal of events takes place.

In micro-organisms, abrupt drops in survival found above a certain cooling velocity (10°C per minute) are associated with the formation of intracellular ice, and the lethality of the intracellular ice depends on the amount of recrystallization occurring during warming, with slow warming producing the greatest

amount (Mazur, 1966, 1967; Sakai and Otsuka, 1967; Sakai and Yoshida, 1967). Mazur (1969) came to the conclusion that, for micro-organisms at least, three main factors influenced the subsequent freezing injury, (a) formation of intracellular ice (b) subsequent growth of ice during warming, (c) adverse effects of solute concentration or of dehydration. The first two are very dependent on the rate of thawing and freezing.

Different rates of freezing and thawing also have varied effects on the subsequent viability of higher plants, though the magnitude of these rates differs from that used with microorganisms. A slow rate for micro-organisms or a tissue section, $1-2^{\circ}\text{C}$ per minute, is a rapid rate for whole plants (Levitt, 1966). With higher plants, subjected rates are usually those found in nature, from $1-3^{\circ}\text{C}$ per hour or less. Slow thawing of whole plants causes no damage (viz. micro-organisms) but rapid thawing appears to cause damage or enhance damage already incurred at an earlier stage (Levitt, 1966). Consideration of rates of freezing and thawing is therefore essential when formulating an artificial freezing test.

As in micro-organisms, intracellular freezing also usually causes irreversible damage in higher plants (Chambers and Hale, 1932; Siminovitch and Scarth, 1938; Levitt, 1956; Olien, 1961). Modlibowska and Rogers (1955) using the technique of cinematography have observed this also in a moss. Asahina (1956) in particular has described ice formation in many plant species and presents an extensive series of photo-micrographs. He found some hardy cells which avoided

intracellular freezing and this confirms the findings of Siminovitch and Scarth (1938) that hardy cells avoid intracellular freezing better than do non-hardy cells. An explanation for this (Levitt, 1956) is the increased cell permeability to water in the hardy cells which must permit a more rapid exosmosis of the intracellular water to the extracellular ice loci. This is also described by Mazur (1969). Even if a plant can avoid intracellular ice formation, quite often damage is caused by extracellular ice formation due perhaps to excessive dehydration of the cell or to other adverse physical effects taking place in the dehydrated protoplasm.

Levitt (1958) comes to the following conclusions.

- (i) The plant does not tolerate intracellular ice formation.
- (ii) Hardy plants are far more capable of avoiding intracellular freezing than are non-hardy plants, presumably due to their higher cell permeability.
- (iii) Hardy plants are more tolerant of extracellular freezing than are non-hardy plants, and the tenderest plants are killed by even the slightest amount of extracellular freezing.

Detailed discussions of frost injury mechanisms and frost resistance hypotheses are given by Levitt (1956, 1966), Parker (1963), Alexandrov (1964) and Mazur (1969).

Experimental induction of frost damage

It is necessary when dealing with frost hardiness to have some method of measuring the plant's ability to withstand freezing con-

ditions. Measurements in the field of damage resulting from a particularly cold winter have been taken as a guide in distinguishing between varieties or species, especially of crop plants (Newton, 1922). Since harsh winters occur only occasionally this is rather a slow method, and because of this, much use has been made of artificial freezing tests. The first workers to use these methods were Harvey (1918) in America and Åkerman (1927) in Sweden. This method has since been adopted by workers all over the world. Peltier (1931) has described his set-up which consisted of three rooms. The first was a controlled temperature greenhouse through which cold air was passed and this hardened the plants. Next there was a freezing room and finally a storage room where the plants were kept at 2°C and subsequently examined for injury. Good agreement has usually been obtained between the rating by such freezing tests and by field survival (e.g. Kneen and Blish, 1941; Worzella and Cutler, 1941; Meader et al., 1945; Amirshahi and Patterson, 1956; Wilner 1960, 1961), but differences may occur because the field conditions are not duplicated by the freezing test. In cases where frost injury occurs mainly in Spring (Till 1956), the order of hardiness then might well be different from that in midwinter or after artificial freezing tests.

These artificial freezing tests deal mainly with whole plants, but separate plant parts have been used recently (Till 1956) and these showed the same frost hardiness as the whole plant. Consequently smaller scale apparatus can be used for these freezing tests.

Frost hardiness must be determined under standard conditions since, as indicated earlier, the frost killing temperature of a plant can be varied by changing the freezing and thawing conditions. Any comparison of different plants therefore requires a rigid control of these conditions.

- (1) The plants must be actually frozen, not merely undercooled.
- (2) Freezing must be at a standard rate.
- (3) A single freeze must be used for a standard length of time.
- (4) Thawing must be at a standard rate.
- (5) Conditions after thawing must be standardized.

Under these conditions, a relatively constant frost killing temperature is obtained for any one variety or species in a specific physiological state.

Till (1956) used the method of Ulmer (1937) and Pisek and Schiessl (1947). He collected the material (leaves or needles) in tin boxes and kept them in a cool place 1-2 hours before placing in the freezing chamber. Natural conditions were maintained by dropping the temperature overnight. The test chamber consisted of a half litre glass jar with a tight fastening in a Dewar vessel, both these being contained in an insulated wood vessel. The temperature was controlled by an alcohol evaporating machine and temperatures less than -18°C could be obtained by adding solid CO_2 . Material was kept at the desired temperature for two hours. Ten of these chambers were usually used at the one time with intervals of $1-2^{\circ}\text{C}$ between treatments depending on the time of year. Thawing was

allowed by draining off the alcohol and leaving for 10-12 hours. Damage was estimated after leaving in water for 10 days in summer, 2-5 months in winter. Cut portions showed no difference to whole plants.

A recent test involves the use of deep freezes (Irving and Lanphear, 1967a). They cut up the material from the pre-treatments and split it into six samples. One sample at 5°C served as a control and the others were placed in styrofoam boxes in a freezer at -6.5°C . When the box temperatures reached -5°C all the boxes, except one, were transferred to a freezer set at -12.5°C . This process was repeated at -17.8°C , -23.5°C and -29.0°C . The rate of temperature drop was 3°C per hour. After two hours at each temperature, the boxes were removed, held at 5°C and the material was allowed to thaw. The samples were then placed in a plastic container under high humidity at room temperature for 36 hours before determination of viability.

Determination and calculation of frost killing point

Both these artificial freezing tests illustrate convenient methods of subjecting plant material to frost, but the main problem in dealing with this subject is how to measure subsequent viability and the method of expressing it.

Older methods of expressing damage involved expressing this damage at one temperature as a percent of healthy plants, and a possible numerical rating of hardiness could then be assigned to particular varieties (Åkerman, 1927). This method is relative and does not permit comparisons between varieties of different species

which may require different freezing temperatures for such a rating. An absolute measurement of hardness is required rather than a relative one.

The simplest method of doing this is to determine the frost killing point, that is the freezing temperature required to kill 50% of the plants (e.g. Schmutz et al., 1961). Other points used have been the "ultimate frost killing point" resulting in 100% killing or the "incipient frost killing point" that just begins to cause injury (Pisek, 1958), but it is now generally accepted that the 50% killing point is the most readily determined and valid point. Although using a standard freezing procedure the methods just described in estimation of injury are subjective.

There are several methods of quantitatively measuring frost injury in plants (Levitt, 1956; Alexandrov, 1964) e.g. depression of photosynthesis, depression of respiration, cessation of protoplasmic streaming, electrolyte release, vital staining, etc. and in the present study it was decided to use both an electrolyte release method and a vital staining method involving the reduction of colourless triphenyltetrazolium chloride.

Osterhout (1922) who principally used seaweeds, developed the electrolyte release method whilst Dexter et al. (1930, 1932) first established the usefulness of the technique in measuring the hardness of plants. They compared the specific conductance of aqueous leachates from frozen and unfrozen samples. Subsequent workers have also used this technique extensively (e.g. Carrier, 1951;

Emmert and Howlett, 1953; Wilner, 1955). Such comparisons are useful, but not strictly quantitative since total electrolytes may vary in different samples. This has been overcome by expressing the amount of cell electrolytes released by freezing as a percentage of total electrolytes released after heat-killing (usually boiling) (Stuart, 1939; Wilner, 1959, 1960, 1961, Wilner et al., 1960; McGuire and Flint, 1962; Cordukes et al., 1966). Since appreciable amounts of electrolytes are leached from unfrozen samples, the calculation involves subtraction of these values from those obtained from frozen samples and is made more cumbersome. Bannister (1970) has cast some doubt on the accuracy of total conductance after heat-killing, however. He found that heat-killed material released less electrolytes than material which had been 100% damaged by drought. He suggested that electrolytes may be bound to the tissue in the heat-killed material and therefore less electrolytes will be leached out.

Various ways of expressing the results obtained have been attempted in the last few years, one of the most satisfactory being that of Flint et al. (1967). This method of calculation has been used during this study, and is described in detail below.

The unfrozen sample is given a value of zero and the heat-killed sample a value of 100. The scale is called the "Index of Injury" and is calculated as follows:

$$I_t = 100(R_t - R_o)/(1 - R_o) ; R_t = L_t/L_k ; R_o = L_o/L_d$$

where

I_t = Index of injury, resulting from exposure to temperature (t).

R_t = Fractional release of total electrolytes from samples exposed to temperature (t).

R_o = Fractional release of electrolytes from unfrozen sample.

L_t = Specific conductance of leachate from sample frozen at temperature (t).

L_k = Specific conductance of leachate from sample frozen at temperature (t) and then heat-killed.

L_o = Specific conductance of leachate from unfrozen sample.

L_d = Specific conductance of leachate from unfrozen sample, heat-killed.

Substituting and simplifying

$$I_t = 100(L_t L_d - L_o L_k) / L_k (L_d - L_o)$$

If samples are uniform then L_k and L_d are identical,

$$\text{i.e. } I_t = 100(L_t - L_o) / (L_k - L_o)$$

After determining I_t for a series of freezing temperatures,

the temperature required to give any selected I_t can be found by interpolation. This temperature can be used as an expression of frost hardness in the absence of a true frost-killing temperature.

The second method used in this study to determine frost injury

is the use of tetrazolium (2,3,5-triphenyltetrazolium chloride or TTC). Colourless solutions of this chemical are reduced to red water-insoluble formazan within the cells of many plants and has been of interest to biologists for almost thirty years. Smith (1951) has reviewed the subject of TTC in relation to enzymes and staining of plant tissue. TTC has since been used qualitatively by many workers in testing for viability of tissue (e.g. Parker, 1953; Brown, 1954; Larcher and Eggarter, 1960; Purcell and Young, 1963; Larcher, 1969), the latter workers distinguishing subjectively between varying degrees of tetrazolium reduction. Recently the TTC test has been put on a quantitative basis by Steponkus and Lanphear (1967). They used samples of a standard fresh weight from material previously subjected to a stepwise freezing procedure, and incubated them in a standard buffered solution of TTC. The formazan formed in the tissue was extracted, compared spectrophotometrically with that formed in the control and expressed as a percentage of the control. By interpolation, absolute values of frost hardness were obtained and tissue survival at a later date was estimated.

Frost hardness in the field

There is a large literature on seasonal variation of frost hardness in the field and its ecological relevance, and reference only will be made to selected papers. Reviews of this subject include Levitt (1956), Biebl (1962) and Parker (1963).

Ulmer (1937) in a thorough study of the hardness of several

species of woody plants, found that species near the tree-line in the Austrian Alps had resistances somewhat different from one another at almost any time of the year. For example Pinus cembra in midwinter could not be killed by -44°C , while Rhododendron ferrugineum was resistant to about -28°C and Vaccinium vitis-idaea resistant to -24°C . In summer they were very susceptible, being killed by temperatures as high as -3°C (Vaccinium vitis-idaea). All the curves paralleled the curve of daily temperatures throughout the year. These findings have been confirmed by Pisek and Schiessl (1947) and Pisek (1950). They both suggested that the species with the lower frost-hardiness in winter are susceptible to winter-killing unless they are covered with snow.

Hardiness may be modified during the coldest months by brief warm spells. Pisek (1950) found this with evergreen trees and Chaplin (1948) with peach fruit tree buds. Damage during winter may not be due to low temperature but to winter drying because of frozen soil causing large water deficits in the plants (Schimper, 1898; Clements, 1938; Michael, 1966). Pisek and Larcher (1954) found that the frost hardiness of Pinus cembra, Rhododendron ferrugineum and Loiseleuria procumbens parallels their drought hardiness and this appears to be quite a common phenomenon. Deciduous trees and shrubs also go through the same seasonal changes, though the increase in hardiness in the autumn is more marked when the leaves fall off (Till, 1956; Parker, 1962).

Daylength as well as temperature has an effect on frost hardi-

ness, short days are more effective in hardening than long days. Moschkov (1935) believed that hardiness of some woody plants is directly influenced by day-length, and Kramer (1937a) indicated that winter killing of Abelia hedges near bright street lights was due to the failure of the Abelia to cease growth and become hardened. Photoperiod appears to be more important at the end of the growing season than at the beginning and long days can prevent hardiness developing (Huystee et al., 1967).

Apart from other environmental factors, another complication to add to an already complex situation is that the hardiness response depends on the developmental stage of the plant (Levitt, 1956, 1966). Young material is much less hardy than more mature material because of an age effect which is independent of any external influences on that tissue. Low temperature treatment has no effect on the hardiness of newly formed buds of evergreens which may survive -30°C during winter (Winkler, 1913), and changes in hardiness may not follow temperature changes at certain times of the year (Ulmer, 1937). Larcher (1969) found that shoots of Quercus ilex show a greater ability to become frost hardened in winter as the tree ages. During the first five winters that hardiness progressively increased, and full capacity for frost-resistance is not reached until the plant enters its reproductive phase. The roots did not change their resistance pattern with age.

All these factors are taken into account in this study of seasonal variation in frost-hardiness of the three Vaccinium species



Plate 9. Freezing apparatus. b: Low temperature bath.
g: Glass containers.
j: Storage jars.

and also an attempt is made to determine if any relation exists with their drought-hardiness. Conclusions are drawn with regard to the ecological importance of variations in frost-hardiness throughout the year.

2. Methods

Cut shoots of the current year's growth of the three Vaccinium species, measuring about 7.5 cm. long (maximum), have been taken from the field and stored overnight with bases in water in closed jars at a temperature of 5°C in the dark. It has been suggested that plants suffering from a water deficit have a greater frost hardiness (Levitt, 1956; Appendix 1), thus all tests have been conducted with saturated plant material.

Artificial freezing procedure

After overnight saturation, external water is blotted off and the samples are subjected to a standard, stepwise freezing procedure. Two Grant low temperature baths are used (Plate 9b), with 70% industrial spirit as coolant. Temperatures as low as -35°C can be attained with temperature fluctuations at any temperature of $\pm 0.1^{\circ}\text{C}$. Rates of cooling and thawing and the post-treatment remained standard throughout all determinations of frost hardiness as discussed in the introduction.

The cut shoots are stored in large jars in one bath (Plate 9j) and subjected to a cooling rate of 2-3°C per hour. This is similar to that encountered in the field. The second, smaller bath is set at the first temperature treatment. When the temperature of the

large bath has reached that of the first treatment, a small sample of material is placed into the glass containers (Plate 9g) and transferred to the small bath as quickly as possible to prevent thawing taking place. The temperature in each container is monitored with a mercury-in-glass thermometer and after equilibration has taken place the material is left at that temperature for $3/4$ hr. After this treatment, the containers are removed from the bath and the material allowed to thaw for ten minutes at room temperature (around 15°C), after which each sample is placed into 25 ml. distilled water in a 25 x 150 mm. heat resistant test tube. The small bath is then set at the second treatment temperature and by the time this temperature has been reached, the large bath, which has been cooling in the duration, has also reached this temperature. A second small sample of material is then transferred to the small bath and the procedure repeated for successively lower temperatures. An untreated control sample of material is taken straight from storage at 5°C and placed in a test tube in 25 ml. distilled water. A typical freezing series is $+5^{\circ}\text{C}$ (control), -4° , -8° , -12° , -16° , -20° and -24°C . Two replicates are taken for each sample at each treatment, and the material is left at room temperature in the distilled water for 24 hours. After this period of time, the damage is estimated by the following two methods, mentioned in the introduction.

Measurement of the 50% frost-killing point

(1) Electrolyte release method

If this method is used, the material is usually cut up immedi-

Table 6. Estimation of t_{50} , *Vaccinium uliginosum* (shoots)

Ben Lui, 1st September, 1969

Electrolyte Release Method

Treatment	Conductance	Total Conductance	Index	Mean
+ 5°C	40	288	0.068	1.800
	54	320	3.533	
- 1.1°C	39	282	0.000	2.760
	50	269	5.521	
- 3.8°C	66	290	10.361	12.028
	71	277	13.696	
- 7.2°C	219	248	86.429	79.176
	188	248	71.923	
-10.2°C	350	370	93.727	90.368
	357	402	87.009	
-13.2°C	308	336	90.329	90.329
	330	360	90.329	
-16.3°C	273	291	92.821	92.821
	-	-	-	

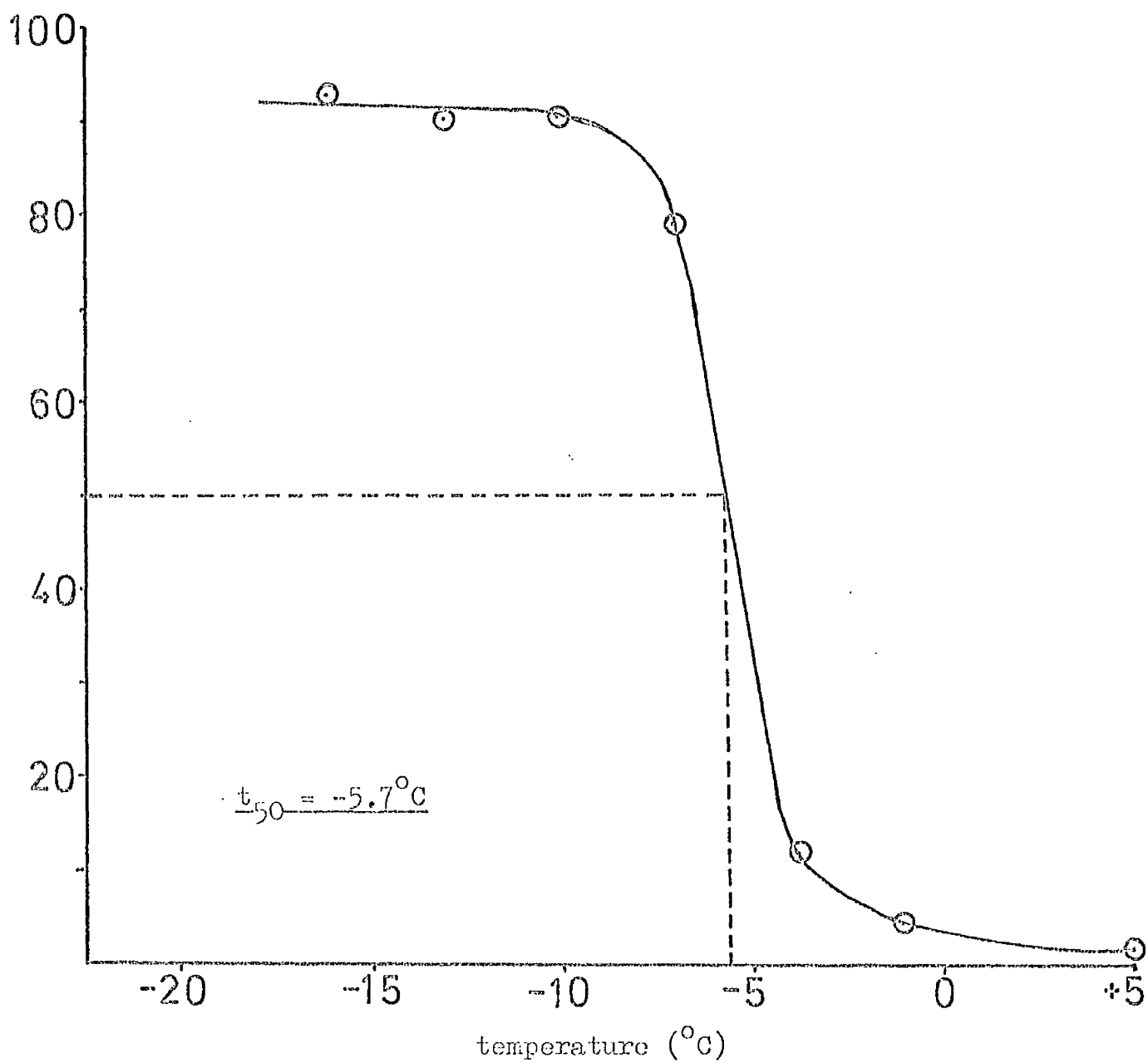


Figure 6. Estimation of t_{50} , *V. uliginosum* (shoots),
Ben Lui, 1st September, 1969.

Electrolyte release method

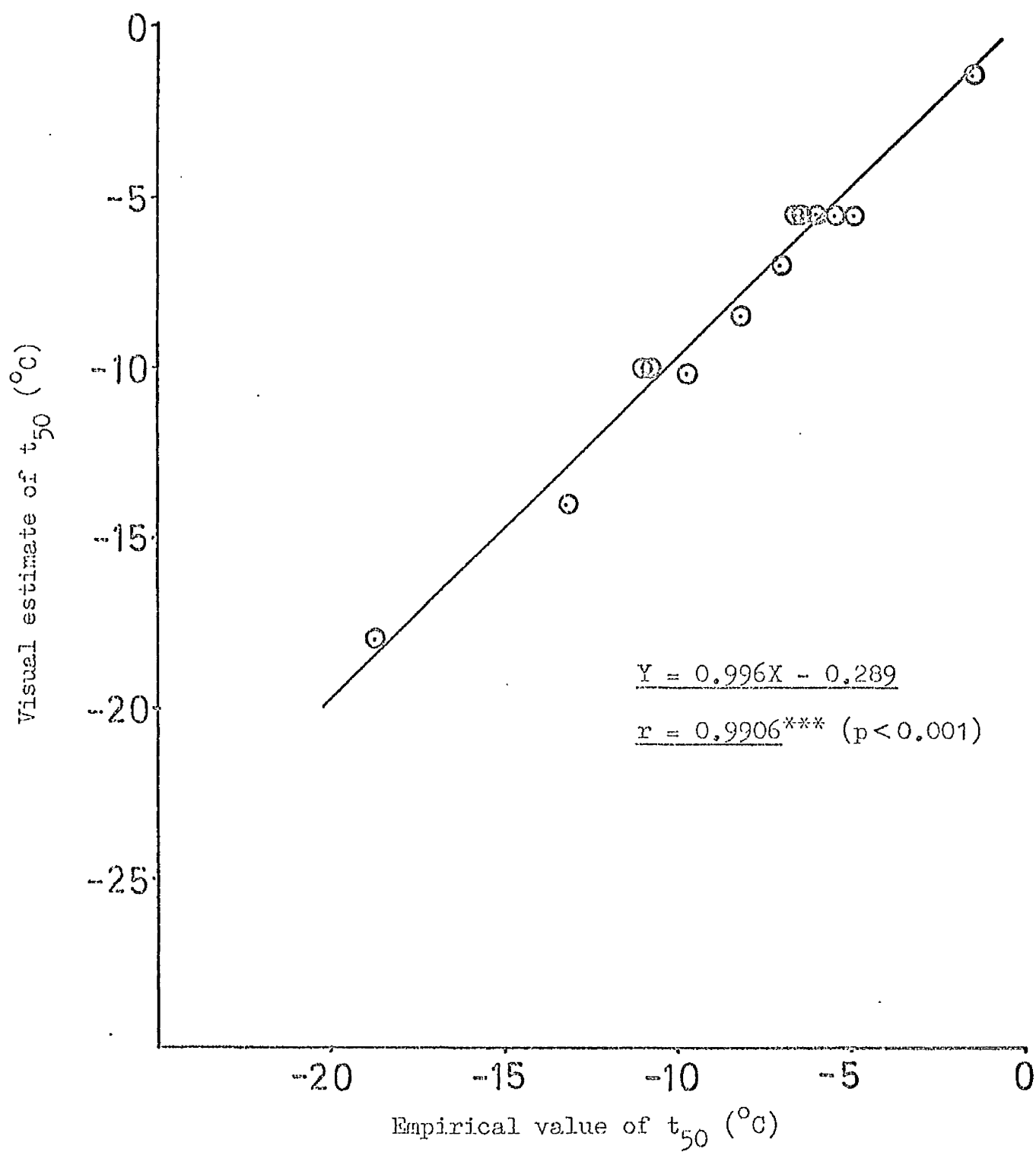


Figure 7. Regression between visual estimates of t_{50} and empirical values of t_{50} estimated by electrolyte release method.

ately before placing in the distilled water but after thawing has taken place. This facilitates the release of electrolytes in damaged tissues. Stem sections are cut to a maximum of 2 mm. in length and leaf sections and whole shoots to a maximum of 3 mm. After 24 hours the conductance of the leachate is measured for each sample. In this study, a Lock conductivity meter is used with a platinum-in-glass measuring cell. Each tube is then covered with aluminium foil, to avoid excessive water loss, and the material is heat-killed by autoclaving. Autoclaving has been found a more convenient method of heat-killing than boiling, and also more efficient since more electrolytes are released by this method compared with boiling (Carpenter et al., 1963). Twenty four hours after autoclaving, each sample is made up to 25 ml. if necessary and the total conductance measured. By using the method of Flint et al. (1967), mentioned in the introduction, an "index of injury" (I) can be calculated for each sample. By interpolation, the temperature at which $I = 50$ can be estimated (Table 6; Figure 6), and this estimate corresponds with subjective estimates of damage* observed just before autoclaving. This empirically derived temperature is considered to be equivalent to the 50% frost-killing point and is designated t_{50} . A regression equation has been calculated for 15 values of t_{50} , correlating empirical values of t_{50} with visual estimates of t_{50} . A highly significant correlation is found, with $r = 0.9906$ significant at the 0.1% level ($p < 0.001$) (Figure 7).

Subjective estimates of damage were made by assessing the proportion of damaged tissue (browned or blackened) on each leaf, and calculating the mean value. This procedure was carried out also when calculating subjective estimates of damage in the drought resistance section (see page 73).

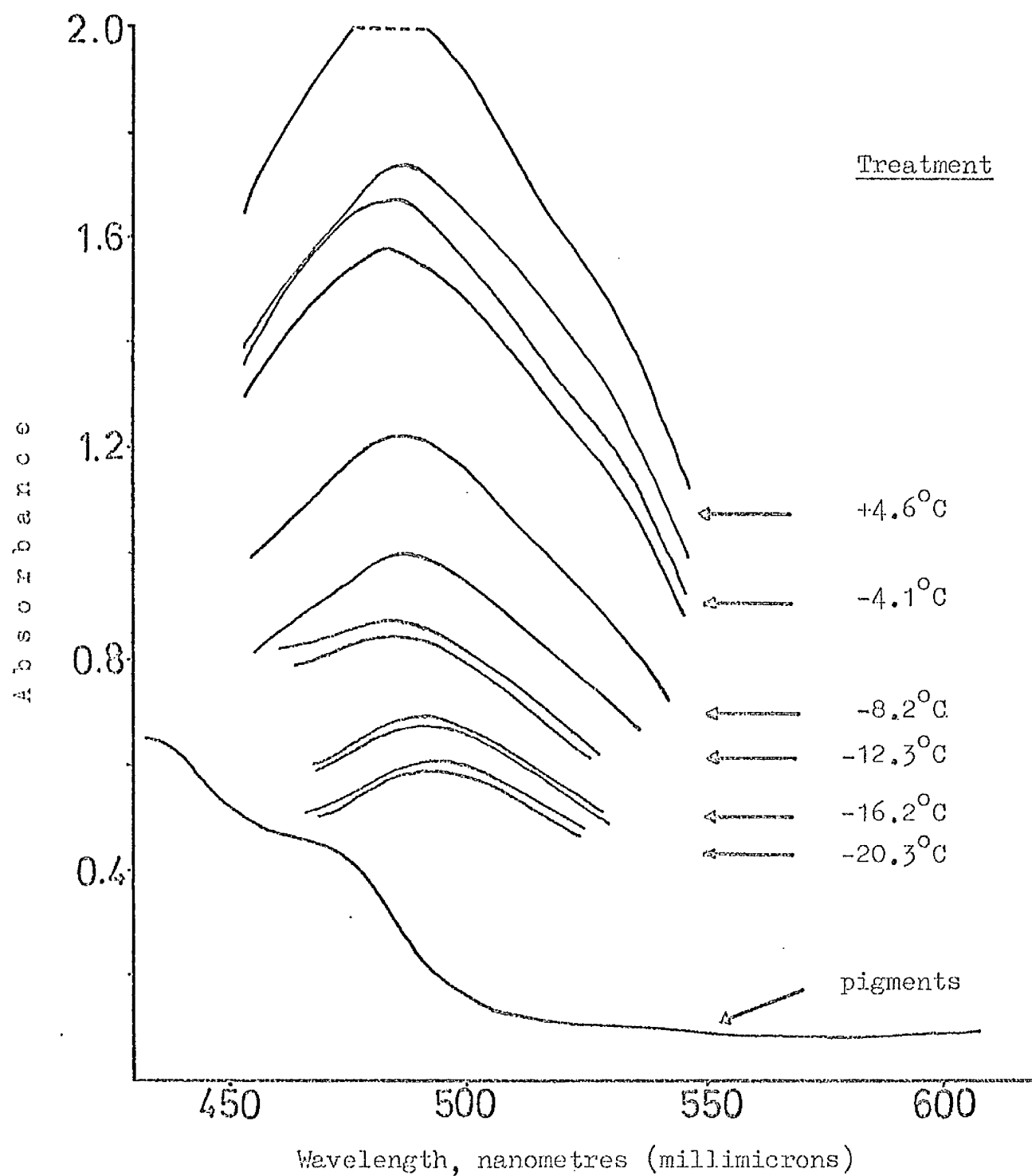


Figure 8. Spectrograph of duplicate formazan extracts for six treatments. V. myrtillus, Ben Lui, 25.11.68

Table 7. Estimate of t_{50} , *V. myrtillus* (stems)

Stiperstones, 21st November, 1968

Tetrazolium Method

Treatment	Absorbance at 500nm	Percentage Absorbance	Mean
+ 4.6°C	1.42	100.00	96.480
	1.32	92.96	
- 4.1°C	1.28	87.32	88.730
	1.24	90.14	
- 8.2°C	1.04	73.24	67.960
	0.89	62.68	
-12.1°C	0.90	55.63	59.505
	0.79	63.38	
-16.2°C	0.53	37.32	36.265
	0.50	35.21	
-20.2°C	0.47	32.39	32.740
	0.46	33.09	
-24.0°C	0.41	28.87	25.700
	0.32	22.53	

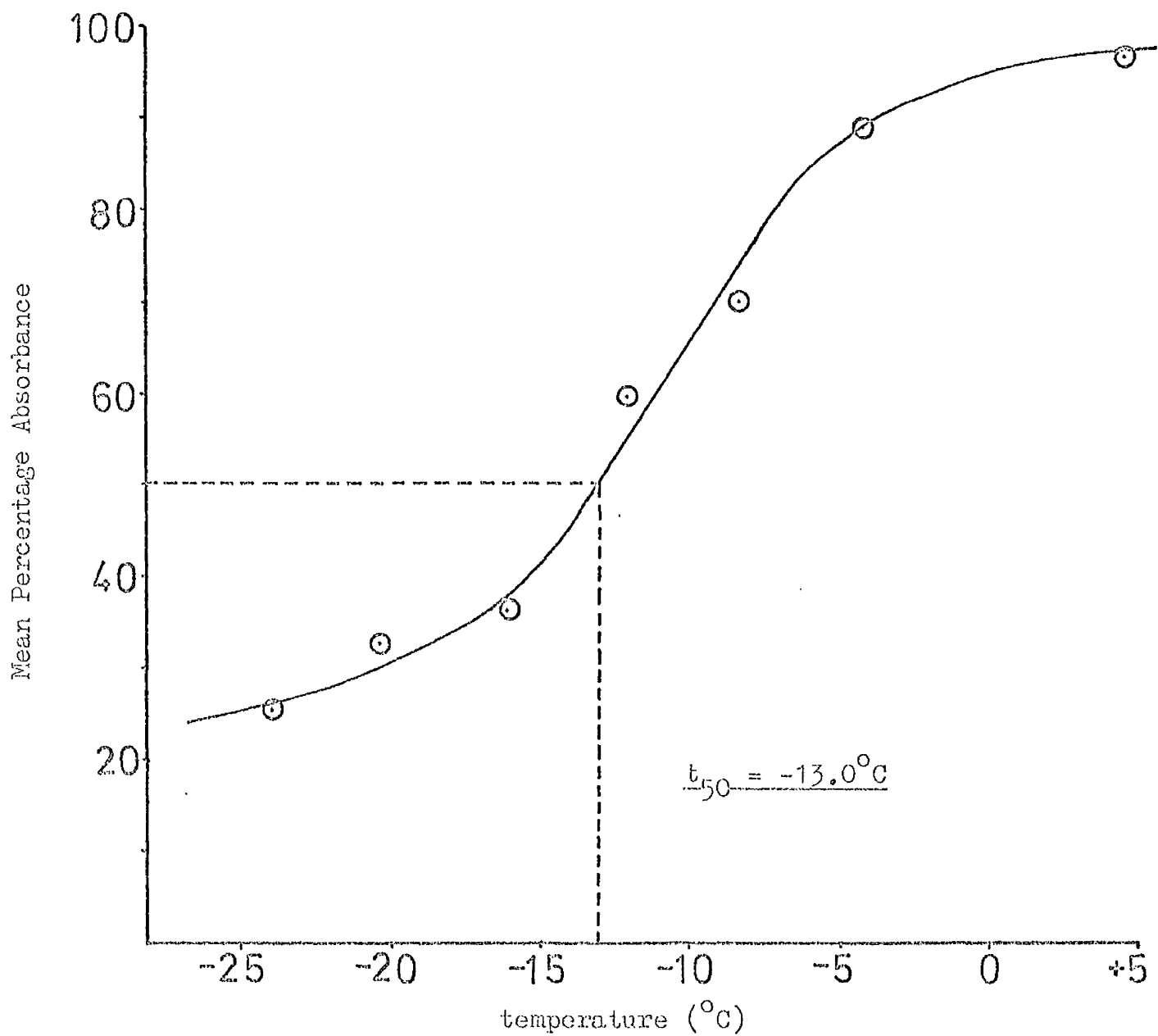


Figure 9. Estimation of t_{50} , *V. myrtillus* (stems)
Stiperstones, 21st November, 1968.

Tetrazolium method

(2) Tetrazolium method - modified from Steponkus and Lanphear (1967).

After leaving whole shoots, whole leaves or whole stems in distilled water for 24 hours after freezing treatment, each sample is cut up and two 60 ± 3 mg. (fresh weight) samples are weighed out. Cut sections are the same size as those in the electrolyte release method. Samples are then placed in 16 x 150 mm. heat-resistant test tubes and to each of these is added 3.0 ml. of 0.6% (w/v) TTC in 0.05M Na_2HPO_4 - KH_2PO_4 buffer (pH 7.4) + 0.05% (v/v) wetting agent (Brij 35). The samples are infiltrated under vacuum for $3/4$ hour and then incubated at 30°C for 15 hours. After this period, the solutions are decanted and the tissue washed twice with distilled water to remove any remaining TTC solution. Five ml., 95% (v/v) ethanol is added to each sample and the formazan extracted by placing tubes in a water bath and boiling for 15 minutes. The extracts are then cooled and made up to 10 ml. with 95% ethanol.

The absorbance of each solution is measured on the spectrophotometer over the range 400-800 nm (m μ). A peak is found at about 485 nm (m μ) which is the wavelength of the absorption peak of reduced TTC (Steponkus and Lanphear, 1967), but as there is interference by other plant pigments at this wavelength (Figure 8), it was decided to use a wavelength of 500 nm (m μ) at which to compare samples. The absorbance of each sample is expressed as a percentage of the control and by interpolation, a value is obtained at which absorbance of treatment/absorbance of control = 50% (Table 7; Figure 9). Usually this was found to correspond with subjective estimates of

damage ($r = 0.8789^{***}$ [$p < 0.001$]) and the value obtained (in $^{\circ}\text{C}$) is considered to be equivalent to the 50% frost killing point. This value will also be designated t_{50} .

Discussion

(a) Electrolyte release method

Negative indices are quite often obtained because less leachates or a greater total conductance occur in one of the treatments as compared with that of the control. In these cases, the sample which has the lowest conductance and the highest total conductance is used as the basis of the calculation (Table 8). This new calculation gives no negatives and only slightly changes the subsequent indices of injury. As the values increase, the error becomes proportionally less and very small.

Table 8. Indices of injury calculated for *Vaccinium vitis-idaea*

Ben Lui, ledge type, 1st September, 1969.

Treatment	A. Index based on control	B. Index based on treatment II	B - A
+5 $^{\circ}\text{C}$	0.000	2.413	2.413
	-1.551	0.899	2.440
-1.1 $^{\circ}\text{C}$	-1.441	1.066	2.447
	-2.467	0.000	2.467
-3.8 $^{\circ}\text{C}$	0.170	2.580	2.410
	-1.394	1.053	2.447
-7.2 $^{\circ}\text{C}$	72.594	72.938	0.670
	78.057	78.586	0.529
-10.2 $^{\circ}\text{C}$	76.594	77.159	0.565
	83.710	84.103	0.393
-13.2 $^{\circ}\text{C}$	84.466	84.841	0.375
	79.411	79.908	0.497
-16.3 $^{\circ}\text{C}$	84.291	84.670	0.379
	82.045	82.478	0.433

It has also been found possible to use small samples of stems (about 50 mg) by placing them in 10 ml distilled water instead of 25 ml. All determinations of t_{50} in stems collected in July, 1969 to January, 1970 have been estimated using this modification.

(b) Tetrazolium method

As with the electrolyte release method, one of the treatments, very often the first treatment below zero, has a higher absorbance than the control. To keep the values between 0 and 100, this treatment has been used (where relevant) as a basis for determining percentage absorbance of the other treatments. This is the reason why the controls very often have a lower value than some of the subsequent treatments.

Quite often it is found that the absorbance of an extract is too great to remain on the spectrograph. In these cases, a small amount of the extract is diluted with 95% ethanol and the dilution factor noted. Errors may be induced if care is not taken with this dilution.

Both the electrolyte release and tetrazolium methods have been found to correspond quite well, 50% frost killing points determined by both methods usually being within 1°C of each other (e.g. Table 9).

Table 9. Comparison of the two viability tests
Vaccinium vitis-idaea, Stiperstones, 31.3.69

Treatment	100 - Index (Mean)	% Absorbance (Mean)
+ 5°C	100.000	96.211
-3.3°C	94.137	98.863
-5.9°C	71.927	82.954
-9.0°C	17.432	26.894
-12.0°C	11.129	36.742
-15.0°C	13.576	21.969
t_{50}	-7.2°C	-7.6°C

Although greater values at the lower temperatures are often found with the TTC test, it is considered that, for statistical analyses and convenience of use, all indices in the electrolyte release method will be subtracted from 100 to equate the results obtained by this method with those from the TTC test. This means that a scale of escape from damage as a percentage of undamaged material is used here.

In this study, no indication will be given that a particular 50% frost-killing point has been estimated either by the electrolyte release method or by the tetrazolium test since -

- (a) the tests have been equated by the method mentioned above,
- (b) it is considered that the t_{50} 's measured by either test are equivalent.

Very occasionally, values have been obtained from which no t_{50} could be estimated since the values ranged only from 100 to 50 compared with the normal 100 to 0. From subjective estimates of damage it was found in nearly every case that a 50% frost-killing point could be obtained if t_{70} was used. However, to compare these sets of data with the normal sets of results, a conversion factor had to be found where $t_{70} \equiv t_{50}$. The following empirical conversion has been found:-

$$\text{Converted value} = \frac{(\text{observed value})^2}{100}$$

The correlation coefficient, $r = 0.9578$, between visually estimated t_{50} values and t_{50} values obtained after conversion is highly significant ($p < 0.001$).

Statistical analyses

(a) Analysis of variance (Appendix 3)

This was used to determine if the variation in frost hardness throughout the year of each species at each site was significant or due to random fluctuations in the population. Comparisons between sites have been attempted using both analysis of variance and t-test methods.

(b) Multivariate regression analysis (Appendix 3)

By calculating the regression of a dependent variable Y on independent variables X_1, X_2, \dots, X_n , the relative influence of each X on the variation in Y can be determined, and also the significance of this influence. The calculation has been done in this study by considering the variation in t_{50} for each sample throughout the year, and determining the influence of at least the two independent variables, temperature and photoperiod, on this variation. Other variables included in some of the analyses are rainfall and age of material.

Calculations involving two independent variables X_1 and X_2 have been carried out on a desk calculator, but more than two independent variables has necessitated the use of a program on the English Electric KDF 9 computer at Glasgow University.

Variation in t_{50} for each site is represented graphically, a distinction being made between different year's growth where relevant. Results of analyses of variance and multiple regression analyses are represented in tabular form.

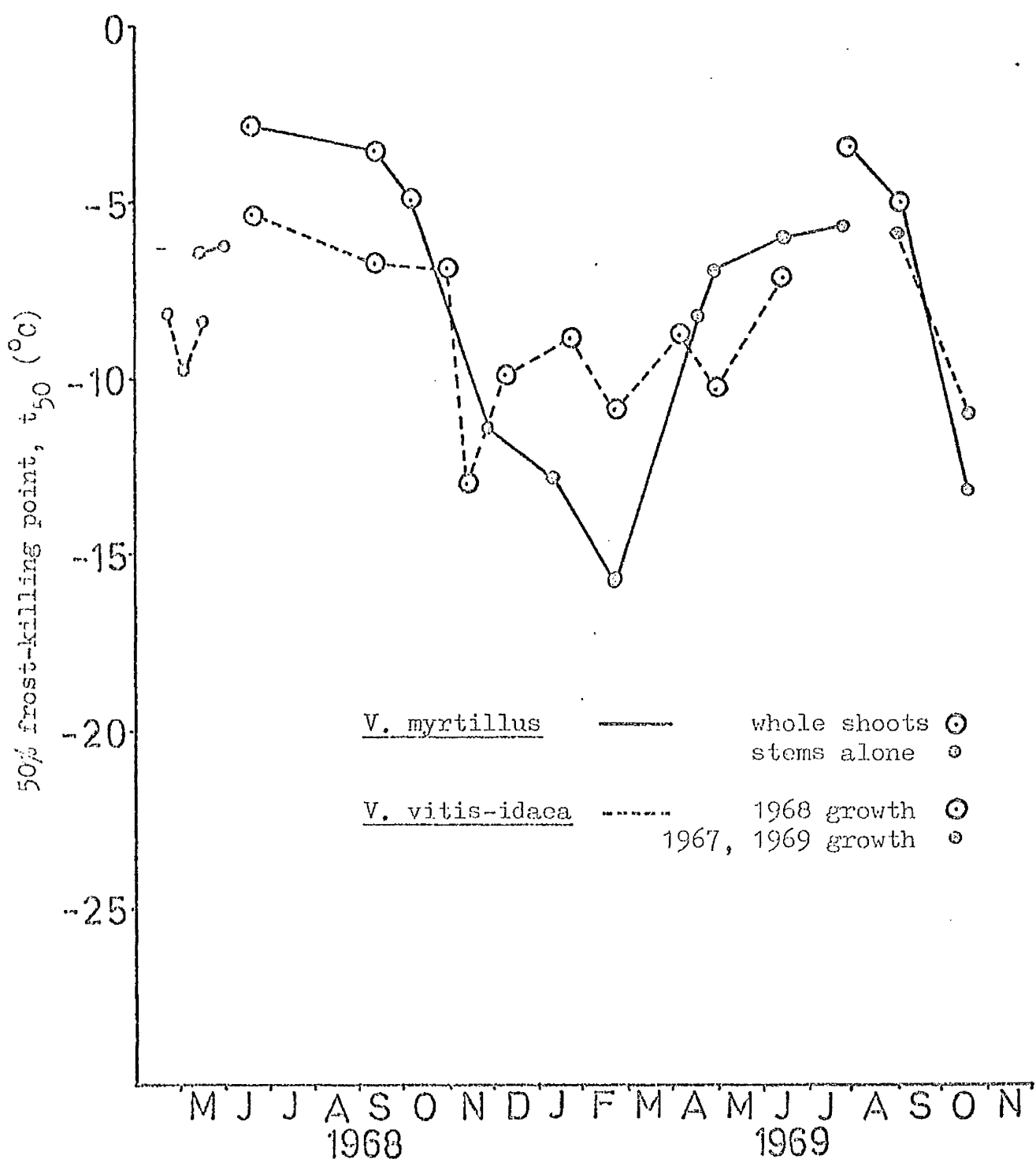


Figure 10. Seasonal variation in frost hardiness,

Ben Lui, ledge forms.

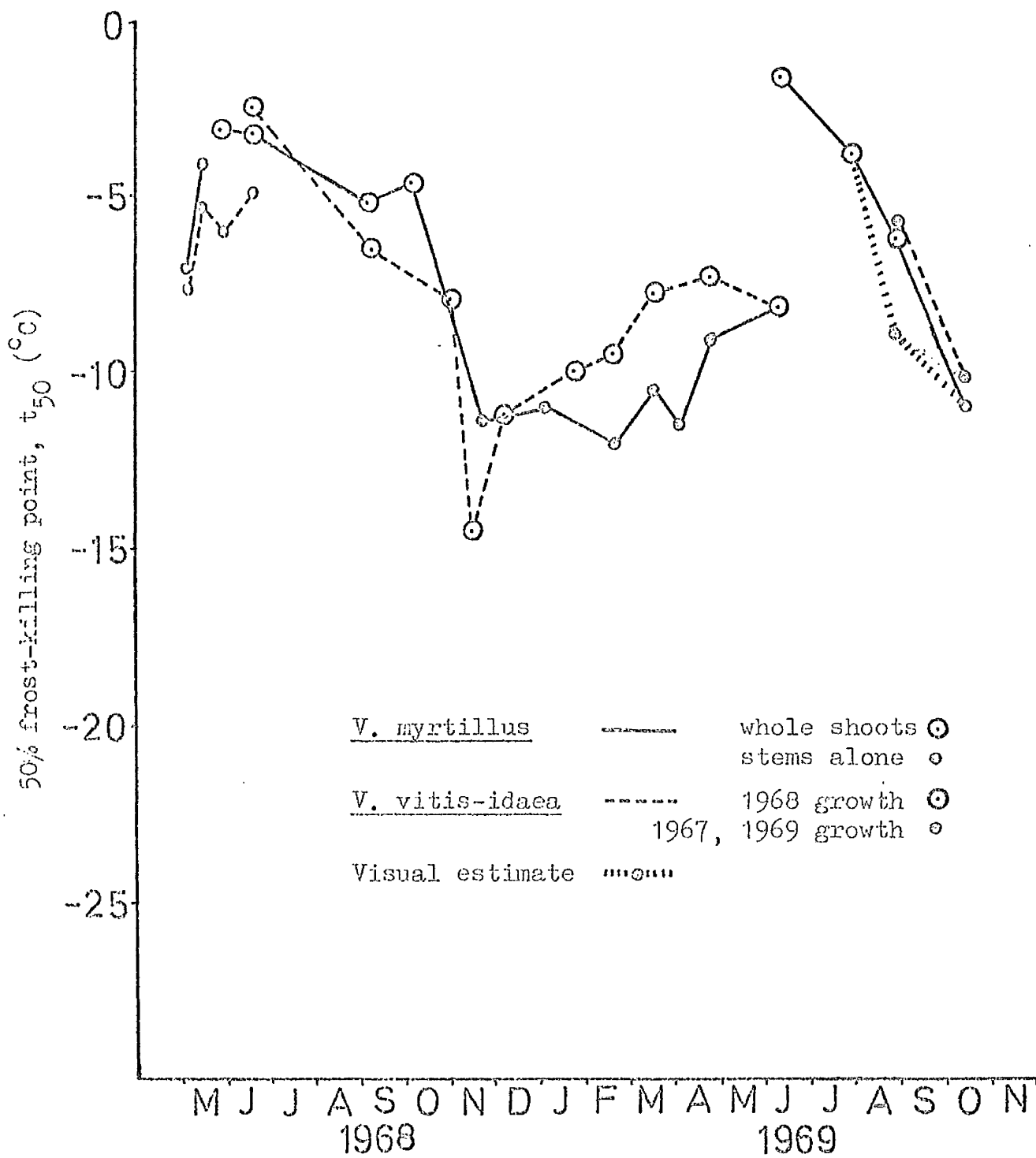
3. Results

Analysis of variance has been carried out on the variation in frost hardness of all samples and an example is given in Appendix 3. In all samples, highly significant differences have been found for the freezing time interaction, i.e. the damage induced by a certain level of freezing varies in determinations done at different times: this difference is equivalent to a difference in t_{50} value. Least significant differences generally indicate a significant difference of about 1°C between t_{50} values in summer and a significant difference of about 2.5°C between t_{50} values in winter within any individual sample.

(i) Ben Lui

Figure 10 shows the variation in frost hardness of ledge forms of Vaccinium myrtillus and V. vitis-idaea.

It is seen that both species have a low resistance in summer and a high resistance in winter, the greatest resistance being in November (V. vitis-idaea) and February (V. myrtillus). The fluctuations in V. vitis-idaea during late winter and spring coincide with fluctuations in temperature (Figure 2), especially in February when there was a period of sub-zero mean temperatures. The sharp drops in Autumn 1968 and 1969 in V. myrtillus are due to abscission of leaves, the stems being more hardy than the leaves by that time of year. As soon as the mean temperature rises above zero in mid-March, large decreases in hardness are seen in V. myrtillus. Frost at night at that time of the year seems to have little effect in maintaining the frost hardness, the increasing daylength coupled with the higher temperatures during the day appearing



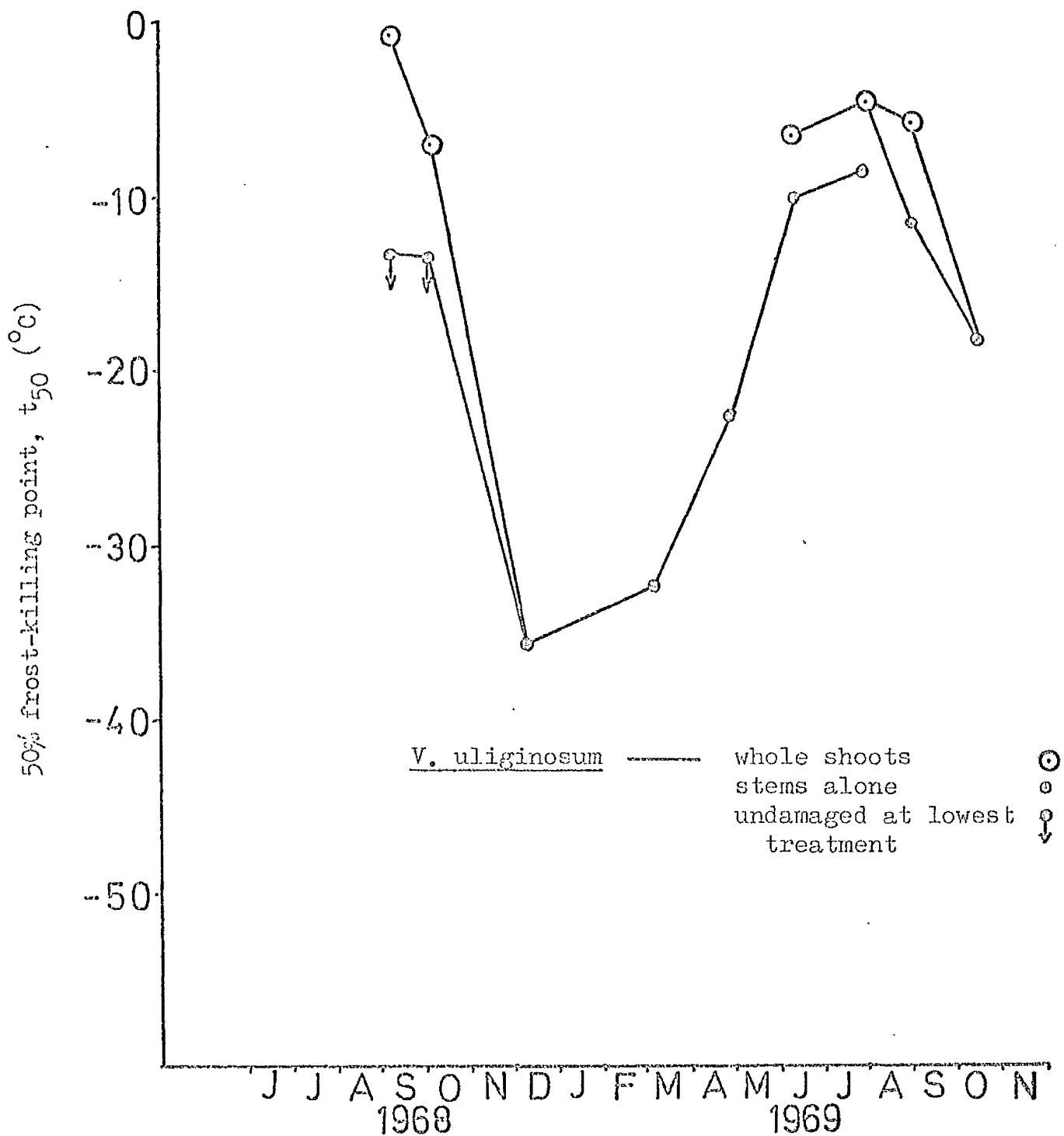


Figure 12. Seasonal variation in frost hardness, Ben Lui.

to have the main influence. Variation in frost hardiness in V. myrtillus at least, seems to parallel the variation in temperature and daylength (Figures 2 and 5), but little distinction between these two factors can be made at this stage.

Figure 11 shows the variation in frost hardiness of the turf forms of V. myrtillus and V. vitis-idaea. Little difference is seen in the hardiness of these forms compared to the ledge forms, though V. vitis-idaea, turf form, has not responded to the low temperature spell in February and appears to lose hardiness earlier than V. vitis-idaea, ledge form.

The young material of V. vitis-idaea, turf form, appears to be more susceptible to frost than that of the ledge form which is about 3°C hardier. The previous year's growth of the turf forms appears to be more hardy in June than the ledge forms and in V. myrtillus, turf form, it is seen that the stems are more hardy than the leaves in August, two months before leaf drop in October.

In Figure 12 is shown the variation in frost hardiness of V. uliginosum. As with the other Vaccinium species, the greatest hardiness is seen in winter and the least in summer, with a maximum susceptibility obtained in September, 1968. Differences between young and old material, and stems and leaves of the same year's growth are seen very clearly. The main difference between this species and the others seems to be its much greater range of hardiness. V. uliginosum, although just as susceptible as the other species in the summer, attains a hardiness of -33°C compared with a maximum of just over -15°C in V. myrtillus, ledge form, and with the other species rarely exceeding -13°C . This ability to harden to a very low

level appears to be a feature of hardy arctic and alpine perennials.

(cf. Billings and Mooney, 1968).

Multiple regression analysis (Appendix 3) has been used to relate four environmental variables (X_1 , X_2 , X_3 and X_4) to the variation in frost hardness (t_{50}) where -

X_1 = mean temperature of the 7 days before date of sampling,

X_2 = mean daylength of the 7 days before date of sampling,

X_3 = age of plant in days from bud-break,

X_4 = mean rainfall (inches per day) for the 14 days before date of sampling,

\hat{Y} = predicted t_{50} value for any specified values of X_1 , X_2 , X_3 and X_4 .

Results obtained using the KDF9 program include regression coefficients, variance ratio for the regression and all sums of squares and products. These have been expressed in Table 10 and percentage variation due to the regression has been calculated ($\frac{\sum \hat{y}^2}{\sum y^2} \times 100$) (see Appendix 3), as well as the significance of the regression from the variance ratio.

Another program, using the Elliott 4100 computer at Stirling University, has calculated the significance of the contribution of each individual factor to the regression. After this calculation for the whole regression, non-significant factors are removed and the regression recalculated for the factors remaining. This is repeated for several levels of significance from 10% ($p < 0.1$) to 0.1% ($p < 0.001$) and the results are presented in Table 11.

Table 10. Regression equations and significance of regression, Ben Lul.

Species	Regression equation	% variation due to regression	Variance ratio	Significance of regression
<u>Vvil</u>	$\hat{Y} = -10.9840$ + 0.1113X ₁ + 0.2741X ₂ - 0.0057X ₃ - 1.7510X ₄	50.70	2.57	Not significant
<u>Vml</u>	$\hat{Y} = -16.2772$ + 0.2125X ₁ + 0.7602X ₂ - 0.0089X ₃ - 2.9893X ₄	78.63	8.28	** (p<0.01)
<u>Vu</u>	$\hat{Y} = -43.2713$ + 1.7841X ₁ + 1.4511X ₂ - 0.0141X ₃ - 2.5065X ₄	84.09	5.29	Not significant (too few readings)
<u>Vvit</u>	$\hat{Y} = -14.1207$ - 0.1318X ₁ + 0.7493X ₂ - 0.0101X ₃ - 1.9642X ₄	71.15	6.17	** (p<0.01)
<u>Vmt</u>	$\hat{Y} = -15.5502$ + 0.1122X ₁ + 0.7122X ₂ - 0.0081X ₃ - 0.2042X ₄	80.21	11.15	*** (p<0.001)

Vvil = Vaccinium vitis-idaea, ledge form

Vml = V. myrtillus, ledge form

Vu = V. uliginosum

Vvit = V. vitis-idaea, turf form

Vmt = V. myrtillus, turf form

Table 11. t-values and significance of individual regression coefficients, Ben Lui.

	Vvil	Vml	Vu	Vvit	Vmt
X_1	0.62	0.88	1.38	0.77	0.51
X_2	1.33	2.66*	1.33	3.37*	3.17*
X_3	0.99	1.30	0.32	1.86	1.29
X_4	0.58	0.67	0.15	0.58	0.05
5% significant ($p < 0.05$)	none	X_2	X_1	X_2	X_2
1% significant ($p < 0.01$)	none	X_2	X_1	X_2	X_2
0.1% significant ($p < 0.001$)	none	X_2	none	X_2	X_2

* = $p < 0.05$

In Table 10, it is seen that the regression is highly significant in three species but not significant in V. vitis-idaea and V. uliginosum. This is probably a true result in the V. vitis-idaea, since the percentage contribution of the regression to variation in frost-hardiness is extremely low, and also no factors are singled out (Table 11).

However, in V. uliginosum, percentage contribution of the regression to variation in frost hardiness is quite high and the non-significance is probably due to the small number of t_{50} values in the regression, causing a decrease in the degrees of freedom and thus a greater residual mean square. In the total regression, no factor is significant in V. uliginosum (Table 11), yet X_1 is singled out down to the 1% level of significance. This apparent paradox infers that there is an interaction between

factors and that the multiple regression analysis may be masking the individual contribution of temperature. From Table 11, it looks as if X_2 (daylength) could be the factor interacting with X_1 (temperature) but by calculating the single regression between these two, a non-significant correlation was found ($r = 0.5507$). The single correlation of X_2 (daylength) and Y (t_{50} value) was found to be significant, however, [$r = 0.7329^*$ ($p < 0.05$)] and this relationship has been masked by the multiple regression. It is thought that although interaction between factors is non-significant, the interaction is sufficiently large to allow this masking to take place.

Accepting that this masking does occur occasionally but that it is possible to recognise it because of the apparent paradox it creates, it is seen from Table 11 that the predominant factor is daylength in V. myrtillus, both ledge and turf forms, and V. vitis-idaea, turf form, and this factor also has the highest t-value in V. vitis-idaea, ledge form, though it is not significant.

Temperature is the predominant factor in V. uliginosum, being highly significant, though as has been stated, daylength is also significant at the 5% level, but has been masked.

Discussion

From earlier analyses, using the two parameters of temperature and daylength only, a difference was found between those Vaccinium species growing on the ledge and those on the turf, the former being more influenced by temperature. Differential snow-cover between the two sites was thought to be a reason for this difference, the turf site being more

insulated from air temperatures. Since the additional results of late summer and autumn 1969 and the extra parameters have been added, the influence of the temperature factor is seen to decrease in all cases except V. uliginosum, with the daylength factor being dominant in the others. It would appear from this that the daylength factor has the greatest influence throughout the whole year but that the temperature factor is effective only at certain times of the year. Insufficient values have been taken to determine the relative influence of these two factors at various times of the year, but the fact that frost hardiness of V. myrtillus, ledge and turf forms, and V. vitis-idaea ledge form, responds to temperature fluctuations in February suggests that temperature may have more influence during late winter and early spring, though it is evident that lengthening days have an effect also.

Tranquillini (1964) has suggested, in addition to the yearly curve of frost resistance being determined by the temperature history of the alpine plant, that this curve may be influenced by an internal annual rhythm. This has seemed likely from the work of Ulmer (1937) and Tranquillini (1958) who both found that dehardening had occurred during the spring in branches which were buried in snow and thus were under constant temperature conditions. Tranquillini (1964) also suggested that the possible effect of changing day length has not been eliminated as a controlling factor.

In all the samples from Ben lui, except V. uliginosum, it has been found that changing daylength is the main factor in controlling frost hardiness, with no evidence of an internal rhythm. Since light can penetrate snow to some depth, the dehardening found by Ulmer (1937) and

Tranquillini (1958) was probably due to changing daylength.

On the Continent, snow cover has been found important in those species which do not harden greatly, including V. myrtillus (cf. Havas, 1969) and V. vitis-idaea, which has been found to harden to only -24°C (Ulmer, 1937). This requirement for snow cover is thought by the latter worker and other Continental workers to be due to a sensitivity to frost by those species at the very low temperatures prevalent in winter in the alpine areas studied. This suggests that, genetically, these two Vaccinium species are able only to reach a certain limiting level of hardening, thus causing limitations to the areas in which they can survive.

On Ben Lui, V. myrtillus and V. vitis-idaea appear to harden only by a margin just great enough to avoid frost damage at any particular time of the winter, but this hardening does not appear to be as great as those Vaccinium species at lower altitudes. This is discussed more fully later.

No difference in hardening is observed between the forms growing on the turf and those growing on the ledge, although it is thought that the turf forms are more insulated from the ambient temperatures by a covering of snow, the larger ledge forms usually protruding through the snow and are fully exposed. This is emphasized by the fact that V. vitis-idaea, turf form, has dehardened to less than -8°C by the middle of March (Figure 11), yet minimum temperatures of -7° to -8°C occurred on four occasions after this date with no observed damage to this form. Snow was observed to lie at a depth of 5 cm. or more from mid-January, 1969 to mid-April, 1969 with only a brief clear spell in February. Thus, snow cover may be essential for survival, even on Ben Lui, especially

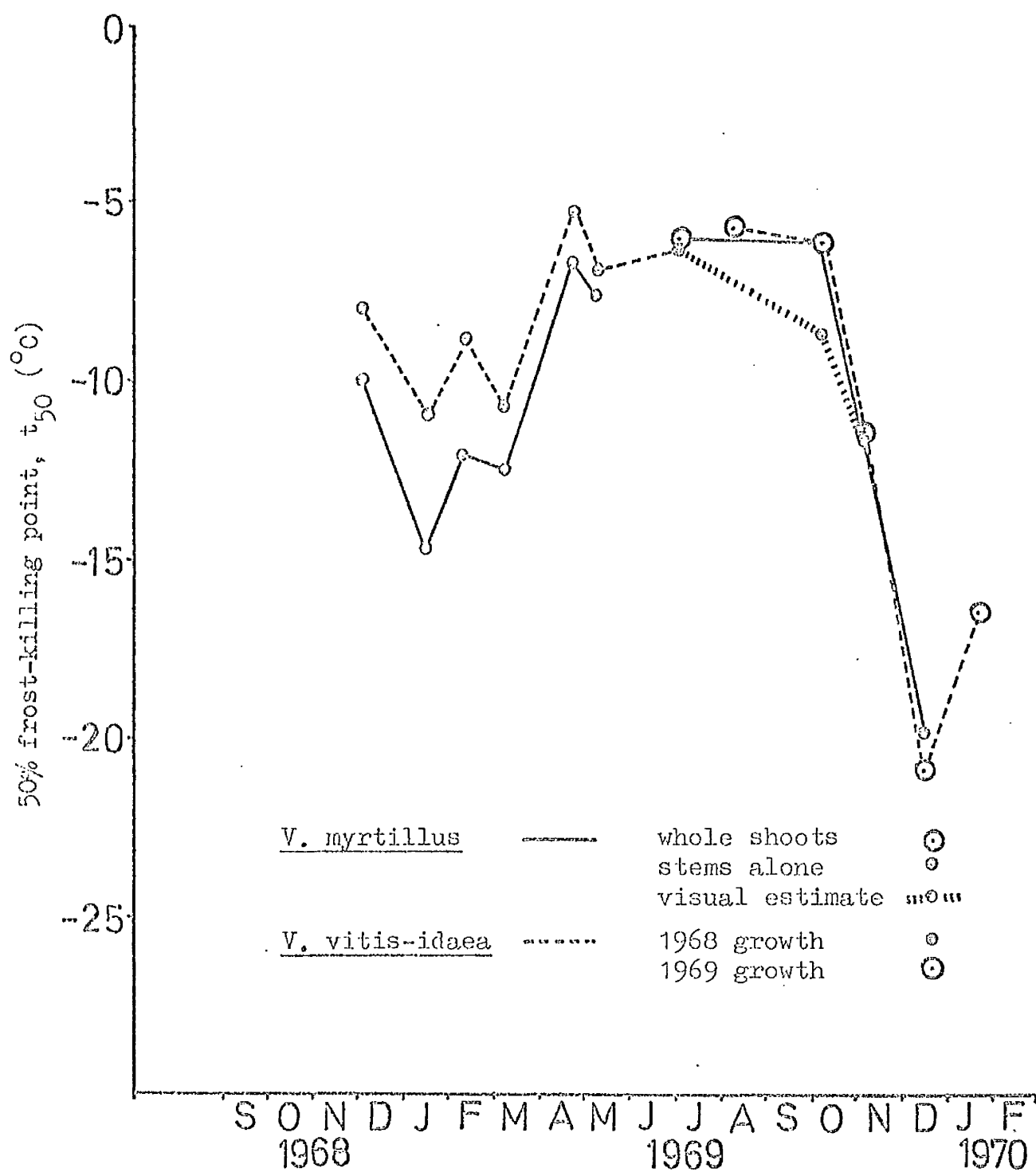


Figure 13. Seasonal variation in frost hardiness, Loch Katrine.

as daylength is the main factor influencing the frost hardiness.

Vaccinium uliginosum, although being partly influenced by changing daylength, mainly responds to temperature.

It must therefore be presumed that when Tranquillini (1964) refers to the yearly curve of frost resistance being determined by the temperature history of the alpine plant, he means a true alpine plant such as V. uliginosum with a large hardening capacity rather than an alpine of restricted capacity such as V. myrtillus or V. vitis-idaea.

(ii) Scottish sites other than Ben Lui

Figure 13 shows the variation in frost hardiness of V. myrtillus and V. vitis-idaea, Loch Katrine. As before, both species show susceptibility to frost in summer and hardiness in winter, especially winter 1969. Frost hardiness is well below minimum air temperatures at any time during winter, especially in V. myrtillus. Both species appear to be fully dehardened by the middle of April before the new buds have expanded. Bud-break has occurred in V. myrtillus by the beginning of April. V. vitis-idaea appears to be less hardy than V. myrtillus during winter 1968-69 but only by a small amount. Variation in temperature and daylength (Figures 3 and 5) appears to parallel the variation in frost hardiness but no subjective distinction can be made between the effects of these two environmental factors.

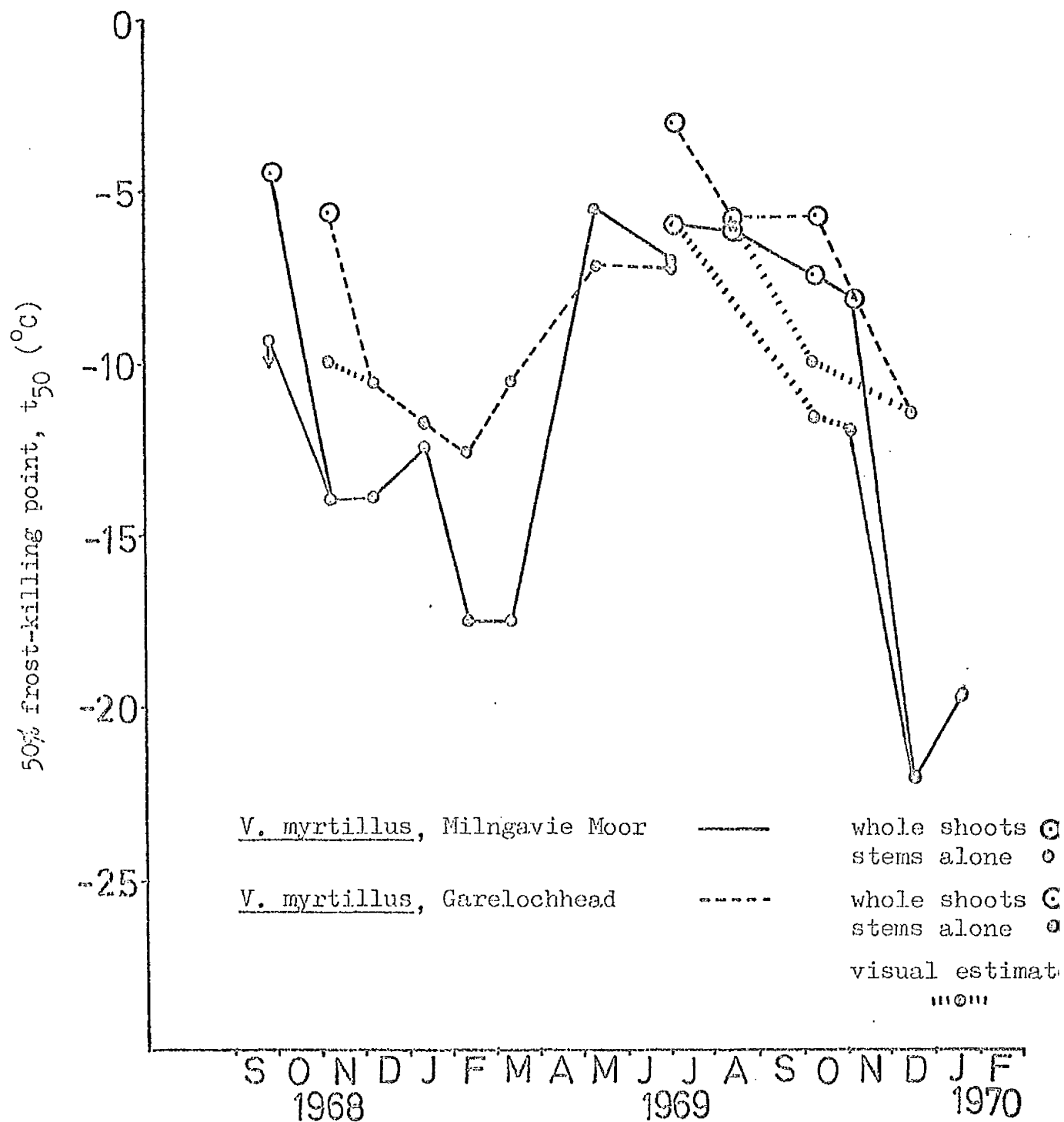


Figure 14. Seasonal variation in frost hardness,
Milngavie Moor and Garelochhead.

Table 12. Regression equations and significance of regression,Loch Katrine, Milngavie Moor, Garelochhead and Malham Tarn

Species	Regression equation	% variation due to regression	Variance ratio (F)	Significance of regression
<u>Vvi</u> Kat	$\hat{Y} = -24.7982$ $- 1.2520X_1 + 2.4186X_2$ $- 0.0384X_3 + 34.9317X_4$	85.73	9.03	* ($p < 0.05$)
<u>Vm</u> Kat	$\hat{Y} = -30.8520$ $+ 0.0748X_1 + 0.9570X_2$ $+ 0.0183X_3 + 24.2919X_4$	84.31	6.72	* ($p < 0.05$)
<u>Vm</u> Miln	$\hat{Y} = -41.0853$ $- 0.1699X_1 + 1.5548X_2$ $+ 0.0199X_3 + 68.9150X_4$	81.99	7.97	** ($p < 0.01$)
<u>Vm</u> Gare	$\hat{Y} = -13.9214$ $+ 0.3363X_1 + 0.3219X_2$ $- 0.0025X_3 + 2.0852X_4$	81.60	4.43	Not significant
<u>Vvi</u> Malm	$\hat{Y} = -13.4039$ $+ 0.0808X_1 + 0.5420X_2$ $- 0.0151X_3 - 0.2349X_4$	88.20	11.21	** ($p < 0.01$)
<u>Vm</u> Malm	$\hat{Y} = -10.3414$ $+ 0.4144X_1 - 0.1414X_2$ $- 0.0161X_3 + 31.6890X_4$	87.44	8.70	* ($p < 0.05$)

Vvi Kat = Vaccinium vitis-idaea, Loch KatrineVm Kat = V. myrtillus, Loch KatrineVm Miln = V. myrtillus, Milngavie MoorVm Gare = V. myrtillus, GarelochheadVvi Malm = V. vitis-idaea, Malham TarnVm Malm = V. myrtillus, Malham Tarn

Figure 14 shows the variation in frost hardness of V. myrtillus, Garelochhead, and V. myrtillus, Milngavie Moor. Although of similar hardness in the summer, the material from these two different habitats show very distinct differences in winter, the form growing on the open moor possessing a much greater hardness. Greater exposure to environmental extremes in the moor habitat is probably the cause of this difference, the material at Garelochhead being very sheltered.

To determine the influence of the same four environmental factors as on Ben Lui, multiple regression analysis was carried out on variation in frost hardness for the material from Loch Katrine, Milngavie Moor and Garelochhead. The results are presented in Tables 12 and 13 in the same manner as for the Ben Lui results.

Table 13. t-values and significance of individual regression coefficients - Loch Katrine, Milngavie Moor, Garelochhead and Malham Tarn

	<u>Vvi</u> Kat	<u>Vm</u> Kat	<u>Vm</u> Miln	<u>Vm</u> Gare	<u>Vvi</u> Maln	<u>Vm</u> Maln
X_1	3.34*	0.22	0.31	1.00	0.54	1.81
X_2	4.64**	2.95*	2.85*	0.83	2.17	0.42
X_3	2.73*	1.38	1.10	0.19	3.06*	1.50
X_4	4.05**	2.54	2.26	0.13	0.03	2.65*
significant $p < 0.05$	X_1, X_2, X_3, X_4	X_2	X_2, X_4	X_1	X_2, X_3	X_1
significant $p < 0.01$	X_2, X_4	X_2	X_2	X_1	X_2, X_3	X_1
% significant $p < 0.001$	none	none	none	none	X_2	none

* $p < 0.05$

** $p < 0.01$

Results indicate (Table 12) that the percentage variation in frost-hardiness due to the regression is high in all cases and significant except in V. myrtillus, Garelochhead. The latter exception appears to be similar to the situation in V. uliginosum (Tables 10 and 11), where non-significance is due to the small number of t_{50} values. Interaction is also seen in Garelochhead material (Table 13) with X_2 (daylength) probably the factor interacting with X_1 (temperature) which has been retained as significant down to the 1% level. Correlation of X_2 and Y (t_{50} value) gives $r = 0.7556^*$ ($p < 0.05$), and regression of X_2 on X_1 , gives $r = 0.6888^*$ ($p < 0.05$). Thus, interaction between temperature and daylength does occur and this causes the multiple regression to mask the contribution of daylength, significant to the 5% level.

From Table 13 (Scottish sites only), daylength appears to be the predominant factor, with the exception of Garelochhead material which appears to be more influenced by temperature. Compared with Ben Lui, these lower altitude samples are also significantly influenced by another factor - rainfall. In V. vitis-idaea, Loch Katrine, the influence of rainfall is as significant as daylength, though the t-value for daylength at the 1% level is greater than that for rainfall. V. myrtillus, Milngavie Moor, is also influenced significantly by rainfall. Although not significant at the 5% level, rainfall contributes to V. myrtillus, Loch Katrine, at the 10% level. It would seem that in these three samples at least, periods of low rainfall have caused significant increases in hardiness. Table 3 indicates that low rainfall in December 1968 and February-March 1969 could have influenced the level of hardiness

in these samples (Figures 13 and 14). From Table 3, it is also seen that rainfall for Ben Lui is 2-4 times greater than the lowland stations for these same months. Altogether, at least twice as much rain falls on Ben Lui compared to the lower altitude stations. It is probable that water shortage rarely occurs at high altitude and no stress will be caused inducing a hardening of the plant.

Vaccinium vitis-idaea, Loch Katrine, appears to be significantly influenced by all the factors. Shading perhaps has modified the day-length effect, allowing the other factors to contribute to a greater extent.

The possibility of shade causing a difference in the effects due to daylight probably contributes in part to the predominant influence of temperature on the Garelochhead material. Even in winter at this site, the light is reduced considerably. In such a stable, sheltered environment it would seem that temperature is able to exert the greatest influence on the frost hardness of V. myrtillus.

Discussion

As with the Ben Lui material, it would seem that low altitude forms are also mainly influenced by daylength, but Garelochhead material appears to be more influenced by temperature for the reason mentioned above. The much lower rainfall at lower altitudes appears to influence frost hardness to a significant level, higher altitudes receiving too great an amount for an effect of water shortage to develop.

One of the main differences between the lower altitude forms of V. myrtillus and V. vitis-idaea (excepting V. myrtillus, Garelochhead) and

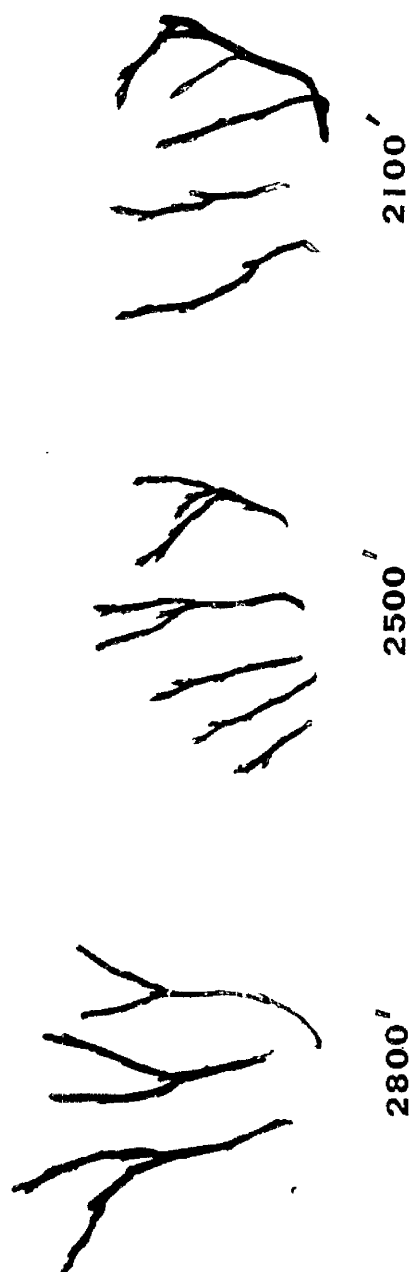


Plate 10. Vaccinium myrtillus collected from several altitudes
on Ben Lui, 19.5.69.

those of higher altitudes is that the lower altitude forms appear to harden to a greater extent in winter. This is surprising especially since temperature extremes are greater at the higher altitude. A probable explanation for this involves the shorter growing season at the higher altitude.

Plate 10 shows shoots of Vaccinium myrtillus collected at different altitudes on Ben Lui on 19th May, 1969. It is seen that although the shoot at 2,800 ft. (850 m) has broken bud, no growth has taken place. An increase in growth with decreasing altitude is also seen. This is due to the decreasing temperature with altitude and it illustrates the shorter growing season caused by lower temperatures at higher altitudes. It has also been observed that leaves of V. myrtillus at higher altitudes turn yellow and fall off earlier in autumn than those at lower altitudes.

The length of time that mean temperatures exceeded 5°C on Ben Lui was 158 days in 1968 and 170 days in 1969 (Figure 2), whereas at Springburn Park this length of time was 216 days in 1969 (Figure 3). This again illustrates the shorter growing season with altitude.

Tranquillini (1967) has suggested that frost-drought damage occurs in Pinus cembra in late winter because of frozen soil and poor transpirational control due to immaturity of the needles. The very short growing season at high altitude was thought to prevent the needles from maturing fully.

Similarly in the present study it is thought that the short season for growth at 2,250 ft. (684 m) on Ben Lui, prevents V. myrtillus and V. vitis-idaea from maturing fully and thus they do not respond fully

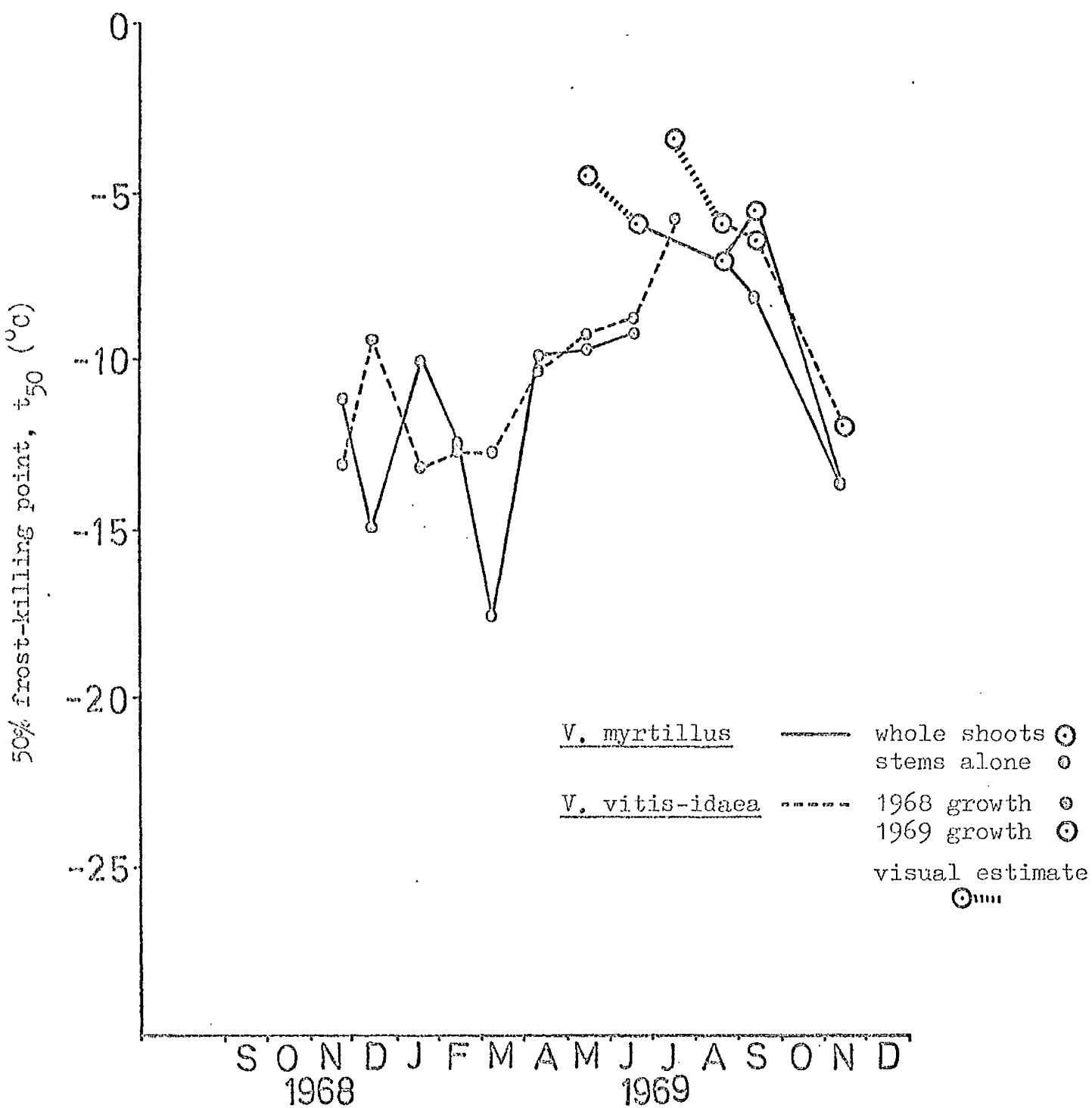


Figure 15. Seasonal variation in frost-hardiness,
Malham Tarn.

to hardening conditions. It has been shown previously (e.g. Levitt, 1956, 1966) that the response to hardening conditions depends on the stage of development of the plant, and it is probable that immature plants do not respond to hardening conditions as efficiently as mature plants.

Vaccinium uliginosum appears to have fully matured in the short season on Ben Lui, and thus is more adapted to that particular habitat.

This hypothesis concerning immaturity due to short season is further substantiated by growth cabinet studies (see later) where after 85 days at 5°C, V. myrtillus and V. vitis-idaea are still continuing to grow whereas V. uliginosum has stopped growing.

It is thought that short seasons at higher altitudes, causing inefficient responses to frost hardening conditions may be the cause of altitudinal limitations in V. myrtillus and V. vitis-idaea. This would probably make snow-cover essential for survival in winter at altitudes even greater than those sampled on Ben Lui. It may be more critical for V. vitis-idaea since this species has not the added protection of leaf abscission in autumn.

(iii) English sites

Figure 15 shows the variation in frost hardness of Vaccinium myrtillus and V. vitis-idaea, Malham Tarn.

As in all the others, the greatest resistance is seen in the winter and the most susceptibility in the summer. The greatest hardness reached in V. myrtillus is in March and the greatest hardness reached in V. vitis-idaea is in January-March and barely exceeds -13°C.

The results of multiple regression analysis are given in Tables 12

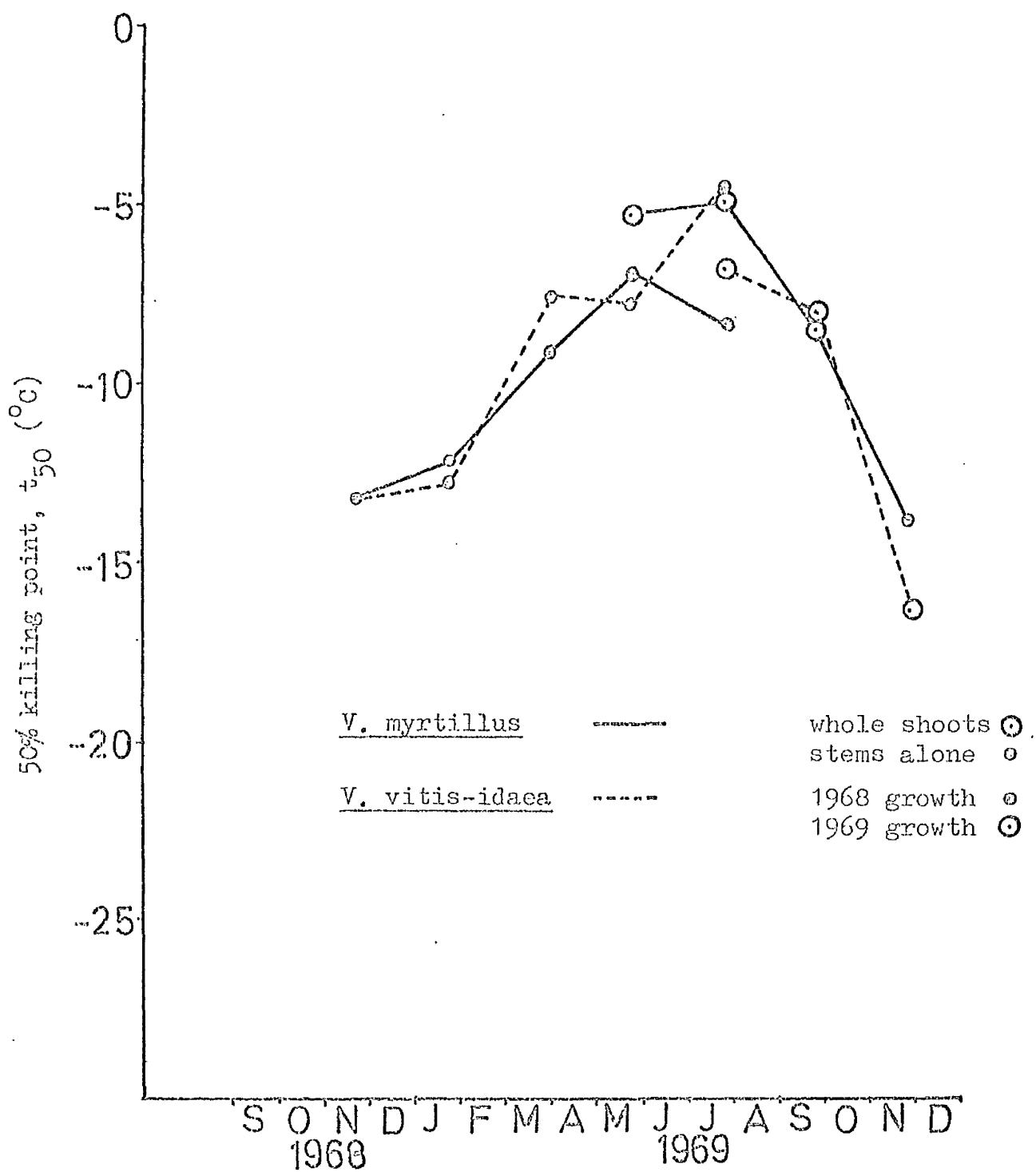


Figure 16. Seasonal variation in frost hardiness,
Stiperstones.

and 13. From Table 12 very high percentage variation due to regression is seen in both species with significant regression in both cases. From Table 13, the factors significantly influencing V. vitis-idaea are daylength (down to the 0.1% level) and age (down to 1% level). The coefficient of the latter is negative however, meaning that the old material is the least hardy. Values show an upward trend (dehardening) from the beginning of the sampling period (Figure 15) and only a few values have been obtained for young material which is very susceptible to frost. The reason for the significant, negative regression with age in V. vitis-idaea is therefore due to most values occurring during the dehardening stage. It does not mean that young material is very frost hardy. It is thought that this factor does not actually contribute to variation in hardness but is more a side-effect of the regression. Daylength therefore appears to be the only factor significantly influencing the variation in frost hardness of V. vitis-idaea.

In V. myrtillus, however, the only significant factor is temperature (to the 1% level), although X_4 (rainfall) is retained at the 10% level.

Figure 16 shows variation in frost hardness of V. myrtillus and V. vitis-idaea, Stiperstones, and Figure 17 of V. myrtillus, Exmoor and Dartmoor. All four of these samples show very similar hardness patterns. Only two environmental parameters, mean monthly temperature and daylength, have been available, and using these, multiple regression analysis has been carried out on the four samples. Results are shown in Table 14. Significance of individual factors has not been calculated because of the very small number of samples in the regression. However, standard partial

Table 14. Regression equations, significance of regression and individual

factors - Stiperstones, Exmoor and Dartmoor

Species	Regression equation	% variation due to regression	Variance (F) ratio	Significance of regression	Individual Factors	
					β	%
<u>Vvi</u> Stip	$\hat{Y} = -17.8630$ - $0.0571X_1 + 0.7381X_2$	79.77	3.94	Not significant	X_1 -0.1375 X_2 0.9654	12.5 87.5
<u>Vm</u> Stip	$\hat{Y} = -19.5550$ + $0.0851X_1 + 0.8342X_2$	99.46	166.86	** ($p < 0.01$)	X_1 0.1639 X_2 0.8826	15.7 84.3
<u>Vm</u> Exm	$\hat{Y} = -26.9120$ - $0.2483X_1 + 1.5590X_2$	99.86	732.02	*** ($p < 0.001$)	X_1 -0.2731 X_2 1.1926	18.6 81.4
<u>Vm</u> Dart	$\hat{Y} = -20.335$ - $0.4762X_1 + 1.1539X_2$	98.97	96.46	*** ($p < 0.001$)	X_1 -0.4643 X_2 1.1632	28.1 71.9

Vvi Stip : Vaccinium vitis-idaea, Stiperstones

Vm Stip : V. myrtillus, Stiperstones

Vm Exm : V. myrtillus, Exmoor

Vm Dart : V. myrtillus, Dartmoor

regression coefficients (β) (see Appendix 3) have been calculated for each factor, compared and expressed as a percentage.

Very high percentage variations in frost hardness due to regressions are seen, and this is due to the very small number of samples in the multiple regression. The variance ratio indicates high significance in all cases except for V. vitis-idaea, Stiperstones, which is not significant. By calculating the standard partial regression coefficients (β) it is seen that daylength has a very marked influence on variation in frost hardness in all samples, temperature only having a small contribution.

The range of frost hardness in those samples from Malham Tarn, Stiperstones, Exmoor and Dartmoor appears to be very similar, with the greatest hardness exceeding -15°C on only two occasions. There is no evidence of immaturity in these samples, even though they do occur at altitudes of 900 ft. (273 m) or greater. The fact that V. myrtillus, Malham Tarn responds more to temperature may indicate that this sample is mature and responding to hardening conditions (viz. V. uliginosum, Ben Lui). It is thought that the hardness developed in these samples is optimal for the conditions under which they are growing, with daylength having the predominant influence except in V. myrtillus, Malham Tarn. The greater length of day at more southern latitudes after the equinox in September (Figure 5) may prevent hardening from fully developing. The longer daylengths before the equinox in March at southern latitudes probably cause early dehardening and breaking of dormancy to occur. This is substantiated by the fact that the more southern samples of V. myrtillus,

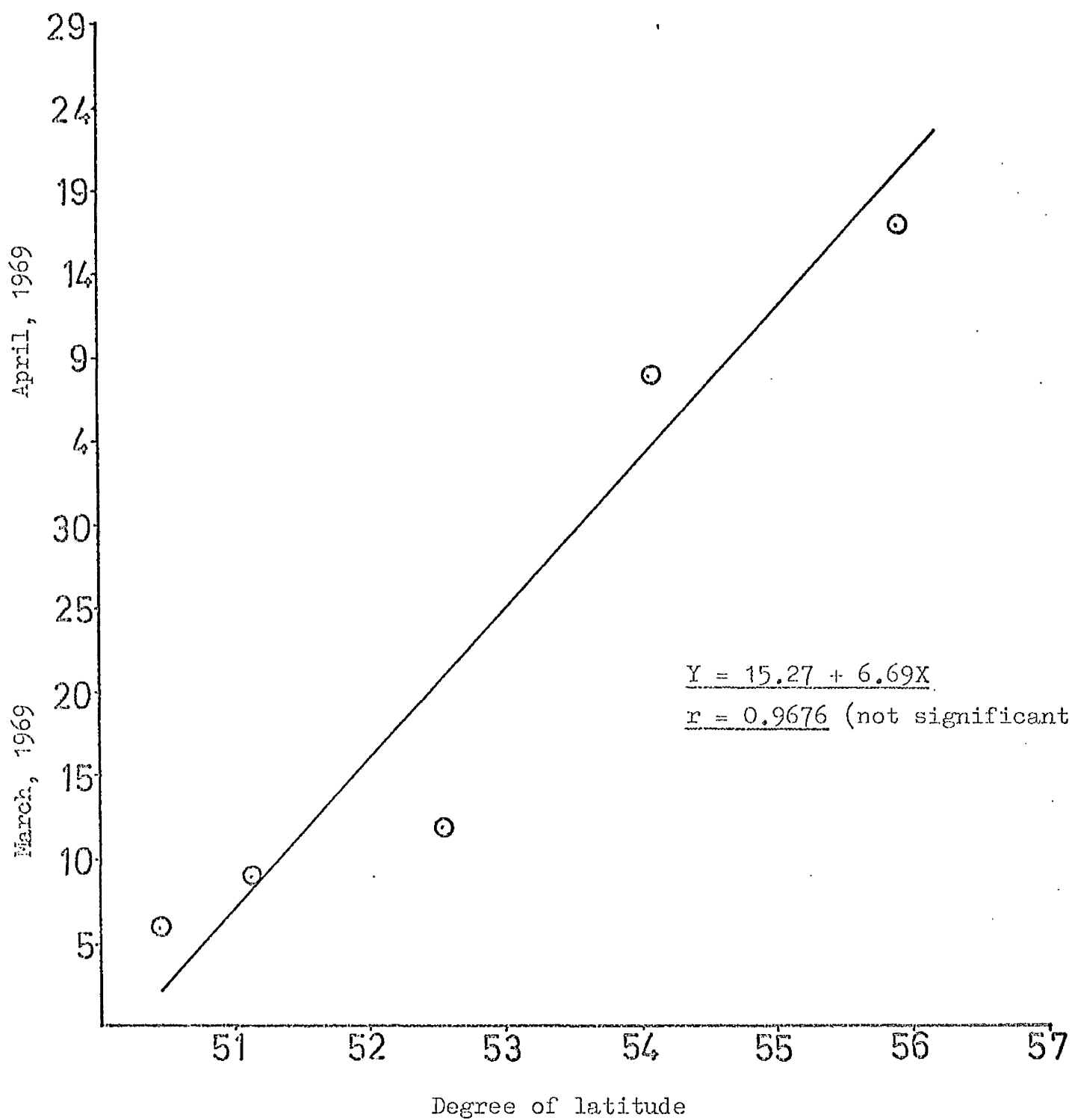


Figure 18. Date of dehardening to -10°C and latitude, *V. myrtillus*.

for example at Dartmoor, Exmoor and Stiperstones, have dehardened to -10°C by the beginning of March, whereas V. myrtillus, Malham Tarn reaches this level at the end of March, and V. myrtillus, Milngavie Moor at the middle of April (Figure 18); $r = 0.9676$ is not significant because of the small number of points. Since mean temperatures at these different latitudes do not differ greatly (Table 2), it is thought that this lengthening of the growing season with decreasing latitude is due to longer day-lengths at certain times of the year at the more southern latitudes (Figure 5). This is supported by growth cabinet studies (see later).

4. General conclusions

From the multiple regression analyses, daylength is found to be the factor most influencing the frost hardiness in nearly all cases, the shorter being the daylength, the greater the frost hardiness. The temperature factor is predominant in Vaccinium uliginosum, Ben Lui, V. myrtillus, Garelochhead, and V. myrtillus, Malham Tarn. Shortage of rainfall at lowland sites in Scotland also significantly contributes to an increase in frost hardiness.

Inefficient hardening caused by immaturity due to short growing seasons at 2,250 ft. (465 m) on Ben Lui, is thought to be the reason for the lesser hardening of V. myrtillus and V. vitis-idaea at this altitude compared with lowland sites, which do not suffer the same environmental extremes. Altitudinal limits of distribution therefore may be caused by the inability to survive frost damage in winter by these two species. Snow cover may be essential for survival at higher altitudes in V. myrtillus and V. vitis-idaea, especially in the latter.

Studies on the frost hardiness from sites at different latitudes indicate an increasing length of season with decreasing latitude and the longer daylengths at more southern latitudes in late September may prevent frost hardiness from fully developing. Both these suggestions are supported by growth cabinet studies.

(b) Drought Resistance

1. Introduction

Water has been known to play an important part in the distribution of many plants for a long time (e.g. Warming, 1895; Schimper, 1898) and on the basis of distribution, three distinct types of plant have been designated. Hygrophytes are plants of habitats with an abundant water supply, xerophytes are those of dry habitats and mesophytes are those in between with an average water supply. These three types show distinct differences in their morphology and their physiology with respect to water relations. It is the purpose of this section mainly to discuss ways in which the physiology of plants is affected by water stress and how, by differing responses to this stress, plants show different distributional and ecological patterns.

Terminology

There appears to be a greater confusion in the terminology of drought resistance than that of frost resistance. The term drought resistance has been most commonly used in an all-inclusive sense to describe all plants suitable for growth in dry conditions no matter what the mechanism of resistance (e.g. Maximov, 1929). Yet there is some tendency to use the term in a more restricted sense. For example, Tumanov (1927) states that drought resistant forms can tolerate permanent wilting without injury for a longer time than non-resistant forms.

Iljin (1930) introduced the term "desiccation resistance" ("Austrocknungs-resistenz") and defined it as the lowest relative

humidity with which the plant can come to equilibrium without suffering damage. Höfler et al. (1941) also used this term, but at the same time preferred the word "Dürreresistenz" for drought resistance. Stocker (1956a) has used the term "protoplasmic resistance" ("plasmatische Dürreresistenz") and this probably should be used only for resistance of the protoplasm itself. Other terms used are "drought tolerance" (e.g. Bayles et al., 1937; Levitt, 1965) and "drought endurance" (e.g. Shanz, 1927). In keeping with the terminology used in the frost resistance section of the present study, drought resistance will be used in a generic, all-inclusive sense of the ability of a plant to remain alive during drought. The ability of plants to survive desiccation will be called "drought hardiness" (Levitt, 1956). Biebl (1962) discusses the terminology in more detail.

Literature

Early work on plant-water relationships is reviewed in Maximov (1929) and Kramer (1949) and many reviews since then have widened our knowledge of the subject. Modern reviews on the subject include those on drought resistance (Levitt, 1956; Parker, 1956, 1968; Stocker, 1956a; Iljin, 1957; Oppenheimer, 1960), water deficits and physiological processes (Stocker, 1960; Vaadia et al., 1961; Henckel, 1964), cell water relations (Slatyer, 1962), water economy and hydrature (Walter, 1955), water stress and plant growth (Kramer, 1963), and determination of water deficits in plant tissues (Barrs, 1968). There have also been many books compiled on this subject e.g. Ruhland (1956), Kozlowski (1964, 1968) and Slatyer (1967). This study will deal only with the aspects of plant water relations which come under the heading of drought resistance, with

particular reference to the ability to reduce water loss and the ability to endure dehydration as factors contributing to survival.

Drought resistance is a quantity that has been measured in many different ways and the data obtained can be evaluated only if there is a clear concept as to whether the method measures avoidance, tolerance or total drought resistance. Avoidance is the ability of the plant to exclude the drought from its tissues and tolerance is the degree of drought within its tissues that it can survive (i.e. drought hardiness). These two aspects are considered in more detail.

Water balance and its measurement

The response of plants with respect to water is directly controlled by the internal water balance which is affected by the relative rates of transpiration and water absorption. No single factor affects the water balance but it depends on the complex of plant, soil and climatic factors. Thus a given response of a plant with respect to its water relations can be measured by determining the internal water balance and this is usually done by measuring the water deficit.

The methods of measuring and expressing water deficits have been and still remain a source of confusion in the field of plant-water relationships. A water deficit is represented by two parameters, the content of water and the energy status of the contained water which is expressed as the total water potential. To describe a water deficit completely, both these should be measured but as water potential is difficult to determine, water deficits are often expressed only in terms of water content. It has been maintained that water content of plants does not give a satis-

factory measure of water deficits (e.g. Kramer and Brix, 1965; Weatherley, 1965) but Macklon and Weatherley (1965) and Barrs (1966) have suggested that this may not always be so when overall gradients are the only factors considered. Relative water content (see later) may be a relatively insensitive measurement of water deficits at high water potential values according to Slatyer and McIlroy (1961), but the relation between the two parameters was not always the same. Until more proof can be obtained, it would seem that water content continues to be a satisfactory way of measuring water deficits, and is used in the present study.

The measurement and expression of water content has been, and still is, a problem in plant physiology when comparisons between plants have been necessary. Water content must be expressed as a fraction or percentage of some basic value. Two of these basic values which have been widely used are oven dry weight and fresh weight.

Dry weight has often been used (e.g. Miller, 1917; Pisek and Cartellieri, 1933; Kramer, 1937b) and still is (e.g. Satoo, 1962; Tranquillini, 1963a; Hatakeyama and Kato, 1965), but it is often unsatisfactory since dry weight may not remain constant either for the short duration of an experiment or particularly for long term seasonal studies. Chaney and Kozlowski (1969) have measured seasonal variations in actual moisture content, moisture content as percentage of dry weight, and dry weight of three types of fruit and found that the percentage moisture content was variously influenced by actual water uptake or loss, change in dry weight or both of these. They concluded that percentage

moisture content alone is not an accurate indicator of internal water balance but must be interpreted in relation to both water weight and dry weight changes of tissues.

Fresh weight has also been widely used as a basis for the expression of water content, but the errors due to dry weight changes are still present (e.g. Halevy and Monselise, 1963), and the extent of changes in actual water content, especially large ones, tend to be reduced (Mattas and Pauli, 1965).

The best available basis for expressing plant water content would seem to be relative to the water content at full turgor.

Stocker (1929) first introduced this technique for measuring and expressing the relative moisture content of leaves and twigs, and made a preliminary survey of variation among species, particularly with respect to drought resistance of plants. He calculated the water deficit (Wasser-defizit) (WD) as,

$$WD = \frac{\text{Saturated weight} - \text{fresh weight}}{\text{Saturated weight} - \text{oven dry weight}} \times 100$$

i.e. the water deficit is the water uptake by a freshly picked leaf as a percentage of the water content of the same leaf when fully turgid.

Since Stocker, many workers have adopted and modified this calculation for their own purpose and have confused the measurements and terminology in this field (e.g. Halma, 1933; Evanari and Richter, 1937; Stålfelt, 1961). Weatherley (1950) developed a new expression and a new method for measuring water stress in field plants. He punched discs from cotton leaves, obtained the fresh weights and floated the discs on water until they became saturated. After oven drying he

calculated the "relative turgidity" of the leaves,

$$\text{Relative turgidity} = \frac{\text{Fresh weight} - \text{dry weight}}{\text{Saturated weight} - \text{dry weight}} \times 100$$

This is the complement of Stocker's water deficit, i.e. $WD = 100 - RT$

Although many workers have used this technique successfully, relative turgidity has suffered the same fate as Stocker's water deficit in being modified (e.g. Fraser and Dirks, 1959). The term "relative turgidity" also has been criticised on the grounds that it suggests some connection with turgor pressure (Walter, 1963; Slavik, 1966) although there is direct little/connection. Weatherley (1965) considers the ambiguity of the term and supports the renaming of the term as "relative water content" (RWC). This latter terminology and its determination will be used in the present study. An error in the measurement of full turgor is that water uptake may continue after full turgor has apparently been attained; but this is fully discussed in Barrs (1968).

Avoidance of water deficits

Slatyer (1955) has shown that the superior drought resistance of grain sorghums over cotton and peanut is due to drought avoidance, and his results point clearly to the two main factors in drought avoidance: resistance to water loss and increased water uptake. By regulating these two factors, the plants can maintain a favourable internal water balance. In the present study only resistance to water loss will be considered with particular reference to stomatal closure as this has been found to be the most efficient method of reducing or preventing water loss (e.g. Stocker, 1956b; Milthorpe, 1960).

The importance of excessive water loss has been recognised since

Schimper (1898) who stated, "Physiological drought is caused by external factors which either reduce absorption or which favour transpiration, or, and this the most frequently, there is a combination of these influences." When investigators measured transpiration rates they found, (unexpectedly), that xeromorphic plants usually had the highest rates (Maximov, 1929). Maximov (1931) attempted to correlate transpirational behaviour with leaf anatomy or ecological status of the plants and failed to determine any pattern. Working with Ericaceous plants, Schratz (1932) also failed to differentiate between different ecological types on the basis of transpiration rates. This failure led Maximov (1931) to conclude "It is not the rate of transpiration when an abundance of water supply is present, but the capacity to restrict water loss to a minimum in time of drought that characterizes the water utilization of the xerophyte."

This type of response to drought stress in plants has been measured by depriving shoots of water and following the decline in transpiration by gravimetric means. Hygen (1951, 1953a, b) has used this method successfully and the method used in the present study is a simplified modification of his technique and calculations.

Following the loss in weight of cut shoots, pretreated to ensure saturation and open stomata, Hygen mathematically analysed his "transpiration decline curves" to obtain several transpiration parameters. He also divided the "transpiration decline curve" into three phases, stomatal, closing and cuticular phases. Using these parameters he successfully distinguished between Vaccinium myrtillus samples from different habitats (Hygen, 1951), and between V. myrtillus, V. uliginosum

and V. vitis-idaea (Hygen, 1953a, b) which were considered to be mesophytic, hygrophytic and xerophytic types respectively.

The computations in this method are laborious and all Hygen's measurements were on a fresh weight basis. Simplified modifications of this technique using saturated water content as a basis of measurement have been used by Pisek and Winkler (1953), Jarvis and Jarvis (1963a, b), Bannister (1964), Lopushinsky (1969) and Hutchinson (1970). These workers, using controlled conditions, extrapolated the linear stomatal and cuticular phases and determined, by the point of intersection, a critical value for stomatal closure. In the present study, the method of Bannister (1964) is used and is described more fully in the next section.

Tolerance of water deficits (drought hardiness)

Although xerophytes are often characterized by striking morphological and anatomical features compared to mesophytes or hygrophytes (e.g. Maximov, 1929; Oppenheimer, 1960), it was soon realised that the ability of plants to endure dehydration was a major factor in their drought resistance (Maximov, 1929; Iljin, 1930). Maximov (1929) states " results indicate that the capacity of enduring prolonged wilting is one of the most important of the characters the sum total of which determines drought resistance in plants. If this is so, the internal physico-chemical properties of the protoplasm would appear to play the principal role in drought resistance, rather than the more superficial morphological or anatomical peculiarities of the plant." This idea has been followed up, for example, by Oppenheimer (1932), Höfler et al., (1941), Levitt (1956)

and Stocker (1956a). Iljin's mechanical concept of drought injury (Iljin, 1930), in which the protoplasmic layer is thought to rupture on excessive dehydration, has been supported, for example, by Höfler (1950) who stated that death results from desiccation more because of mechanical causes which are dependent on the construction of the cell wall, the thickness of the wall coatings and the size of the vacuole than upon the inner characteristics of the plasma as such, but he stressed that it is difficult to distinguish between the resistance of an entire cell and that of its protoplasm alone. This latter difficulty has probably contributed to the formulation of other hypotheses of drought injury and hardness, reviewed in Levitt (1956, 1958), Parker (1956, 1968) and Stocker (1956a). It is thus probable that the atmospheric desiccation method, so commonly used to test tissue, measures the entire cell and perhaps tissue hardness, instead of the hardness of the protoplasm alone.

Measurement of drought hardness

This will be discussed only with reference to the method used in the present study - water absorption capacity after droughting to various water deficits. Other methods of measurement are reviewed in Levitt (1956), Parker (1956) and Stocker (1956a).

Using the method of Stocker (1929) for measuring water saturation deficits, Oppenheimer (1932) and Höfler et al. (1941) have developed a technique of measuring the "sub-lethal" water saturation deficit in the former, and the "critical saturation deficit" in the latter case.

Oppenheimer (1932) found that when 5-10% of the leaf tissue was covered

with necrotic spots, the "sub-lethal" water deficit had been reached.

Rewatering and measuring damage substantiated this. Höfler et al.

(1941) determined the water deficit at the drought killing point where,

$$\text{critical saturation deficit} = \frac{\text{saturation weight} - \text{fresh weight at critical point}}{\text{saturation weight} - \text{dry weight}} \times 100$$

These procedures have been used successfully, for example by Pisek and Berger (1938), Rouschal (1938) and Pisek and Winkler (1953). Recently Pharis (1966) and Pharis and Ferrell (1966) have used lethal needle moisture content (% RT) as an indicator of damage to conifers, and have determined differences in drought hardiness. Bannister (1970) has used lethal moisture contents to determine differences in hardiness of Vaccinium myrtillus.

Oppenheimer and Mendel (1934), however, working with leaves of Citrus sinensis, found that no necrotic spots appeared as an indication of "sub-lethal" water deficits, but the leaves just gradually lost their colour. They considered the water-absorbing capacity of damaged, wilted leaves and suggested that the degree to which the water-absorbing capacity is lost may give useful information about the amount of injury suffered at wilting.

Arvidsson (1951) has surveyed the critical water saturation deficits of many plants on the Baltic island of Öland, and has also used, to a certain extent, the criterion of water-absorption after droughting to determine injury. He found that the water-absorption capacity was reduced before drought damage appeared, and that the amount of damage and

the reduction in water-absorption capacity appeared to be quite well correlated. In Convallaria majalis and Maianthemum bifolium, he found that when 5-10% of the leaf tissue had been damaged, the level to which the leaves resaturated after replacing them in the moisture chambers was 85-95% of the saturated water content.

Recently, Oppenheimer (1963) has discussed the technique of measuring resaturation deficits after wilting in several Mediterranean evergreen trees and shrubs. He found that serious damage in detached leaves set in when about 35% of the water content at saturation is lost. Less wilted leaves were found to approximately regain their initial weights on rehydration. This point, above which leaves regained their initial weight but below which a deficit remained in the leaves after rehydration, Oppenheimer called the "permanent turgor loss point" (PTLP). Below this point he found the number of damaged cells increased steadily, producing an increased "water resaturation deficit" which was more or less linearly related to water loss beyond the PTLP.

Rychnovská-Soudková (1963), Rychnovská and Květ (1963, 1965) and Rychnovská (1965) have studied the reversibility of the water saturation deficit as one of the methods of causal phytogeography of different species of grass. They found (Rychnovská and Květ, 1965) that a continental type of grass (Stipa) fully recovered from water deficits over quite a large range but that irreversible changes occurred beyond a certain deficit. But with an oceanic type (Chrysopogon) they found that an increase in water deficit brought about an increase in the water-holding capacity of the leaf tissues but irreversible changes also took

place. They considered that the former type would seem advantageous to long rainless periods but the latter type would seem to favour meso-phytic conditions (oceanic) where only short periods of drought have to be survived. Bannister (1970) has also used resaturation responses to distinguish successfully between different types of heath vegetation.

In this present study, this resaturation method is used as an indication of the tolerance of the Vaccinium species to desiccation, i.e. as a measure of their drought hardness.

Field studies

As with frost resistance, the literature on drought resistance is immense and only selected papers with reference to heath and alpine plants will be considered here. Other papers are reviewed in Pisek (1956), Stocker (1956b) and Biebl (1962).

Many of the early studies of the water relations of heath and alpine plants and their ecology (e.g. Huber, 1924; Stocker, 1923, 1931; Firbas, 1931; Pisek and Cartellieri, 1933; Michaelis, 1934; Cartellieri, 1935; Ulmer, 1937; Pisek and Schiessl, 1947) have been concerned with seasonal transpiration rates, osmotic potentials, natural water deficits and seasonal critical water deficits or critical water contents.

Biebl (1962) and Tranquillini (1963) have both cited Larcher (1957) who has gathered together all measurements of natural water content by Pisek and his co-workers, carried out near Innsbruck during several years from January to April. Larcher (1957) has compared these findings to the annual trend of resistance to dehydration measured by Pisek and Larcher (1954). Natural dehydration was seen to be at its maximum

between February and April with the water content of mature Pinus combra and Picea excelsa * needles and of the leaves of Loiseleuria procumbens remaining well above the level of dehydration injury. However, the water deficit of leaves of Rhododendron ferrugineum was seen to exceed the critical limit on several occasions. It was thought that the differences are due to different requirements for snow cover. Mature pine and spruce would appear to survive winter drying in snowless environments below a limiting altitude but Rhododendron seems to have a dependency on snow cover and grows only in such places which are covered with snow reliably and for a long period in winter. The rhizomatous habit of Loiseleuria, making more use of melted surface water, was thought to contribute to its survival in snowless areas. These differences in response to desiccation and snow cover have explained differences in vegetational composition in the alpine areas studied.

Tranquillini (1967) has confirmed the hypothesis of Michaelis (1934) that immaturity of needles due to the short growing season contributes to greater frost-drought damage and is probably a large factor in determining timber and tree lines in West Tyrol.

He suggests that because of the short growing season at that altitude, controlled by decreasing temperature, the needles of Pinus montana * and Picea excelsa * are unable to mature fully. Thus they cannot control transpiration efficiently, with the result that larger water deficits develop, especially since water absorption is impeded by deep frozen soil on sites with only light snow cover. The older more mature needles

* see Appendix 2

appeared to be more drought resistant than the young needles (Tranquillini, 1967, Plate 2).

Hygen (1953a) studying the water relations of three Vaccinium species, has found very pronounced differences with respect to transpiration. He has found that the xerophytism exemplified in Vaccinium vitis-idaea is associated with -

- (a) a comparatively low transpiration rate in plants with well open stomata,
- (b) a very rapid closing reaction as a response to water loss,
- (c) a great percentage reduction in transpiration rate by closing of the stomata resulting in
- (d) an extremely low cuticular transpiration rate.

The comparatively hygrophytic Vaccinium uliginosum he showed to have the opposite characters in all these respects, while V. myrtillus (mesophytic) showed characters in between these two.

Bannister (1964) has shown that the characteristic vegetational pattern of Erica cinerea, E. tetralix and Calluna vulgaris which grow in dry, wet and intermediate habitats respectively, can be explained by the responses of these plants to water stress. Stomatal closure points of E. cinerea and E. tetralix were found to be significantly higher than those of Calluna. However, when comparing stomatal closure points of Calluna from dry, moist and wet habitats, it was found that there was a significant difference between dry and other sites but not between moist and wet, the lowest closure points being found in the dry habitat. It was suggested that Calluna from drier sites is adapted to that particular

habitat not by greater transpirational control but by an ability to forgo a degree of stomatal protection for the sake of continued assimilation. Stomatal closure points have thus been shown useful in differentiating between different species and also reflect the environment in which the plant has been growing.

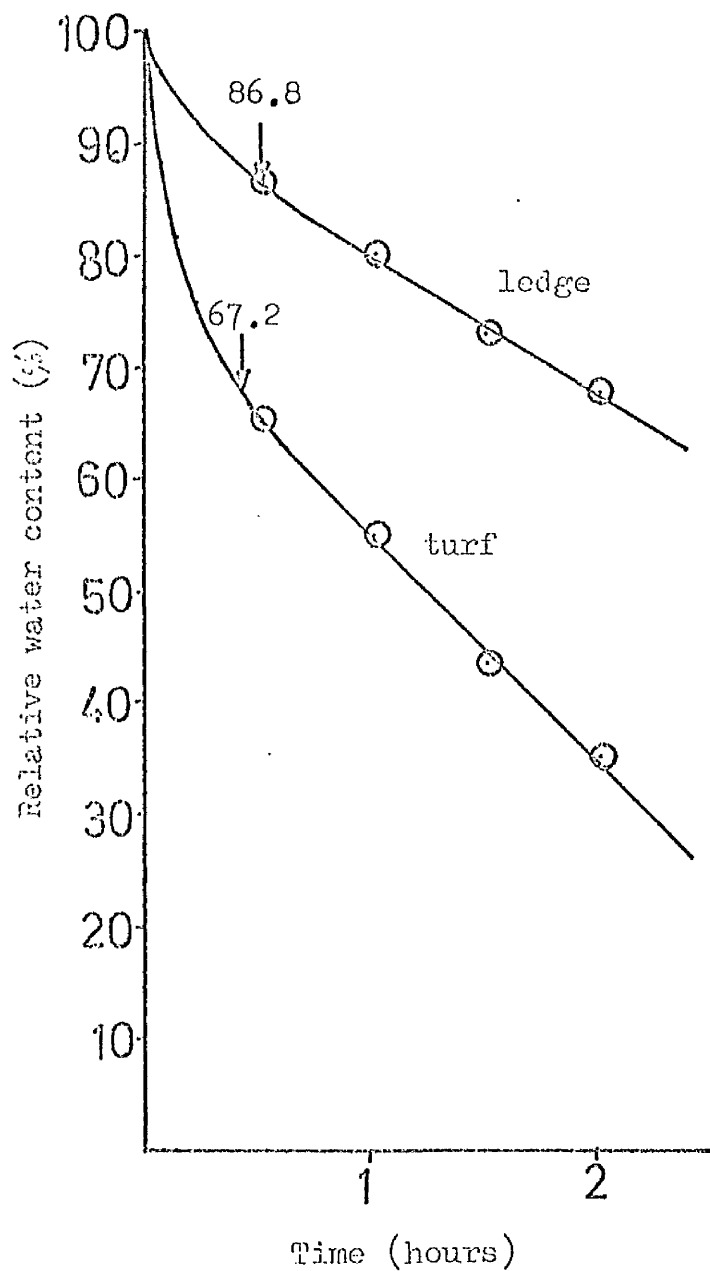
Similar conclusions are found for the transpiration of plants growing in situ (Rychnovská and Květ, 1963). The most xerophytic form (Festuca dominii) showed no control of transpiration whereas the more oceanic Corynephorus showed a much greater control.

With many of these points in mind, the water relations of the three Vaccinium species are followed in the present study with respect to different responses at different times of the year, and these responses related to the habitat differences between and within species.

2. Methods

Material is brought into the laboratory from the field and individual shoots are stood in water in closed 75 x 16 mm polythene tubes. Few measurements have been taken of fresh weights in the field since it was not possible to standardise the time of day at which collections were made. Because of this, any seasonal variation in relative water content (RWC) which might have been found would be difficult to distinguish from diurnal variation in RWC. After overnight saturation in the dark at room temperature, with a short period of 3-4 hours daylight in the morning, each shoot is removed from its saturation chamber, blotted dry and weighed. The saturation treatment and short period of light are thought to induce wide-open stomata (Hygen, 1951).

(a) Moisture loss



(b) Rate of moisture loss

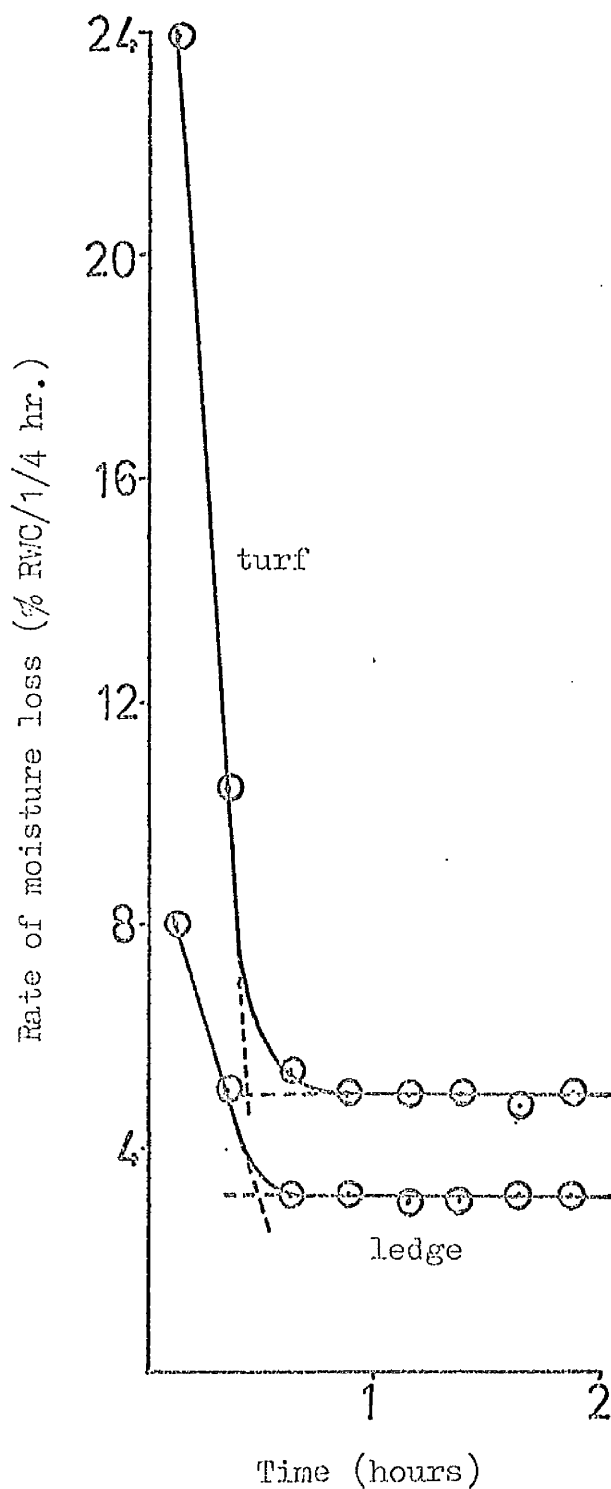


Figure 19. Determination of stomatal closing point, *Vaccinium vitis-idaea*, Ben Lui, ledge and turf forms, 3-4-69.

Measurement of stomatal closure

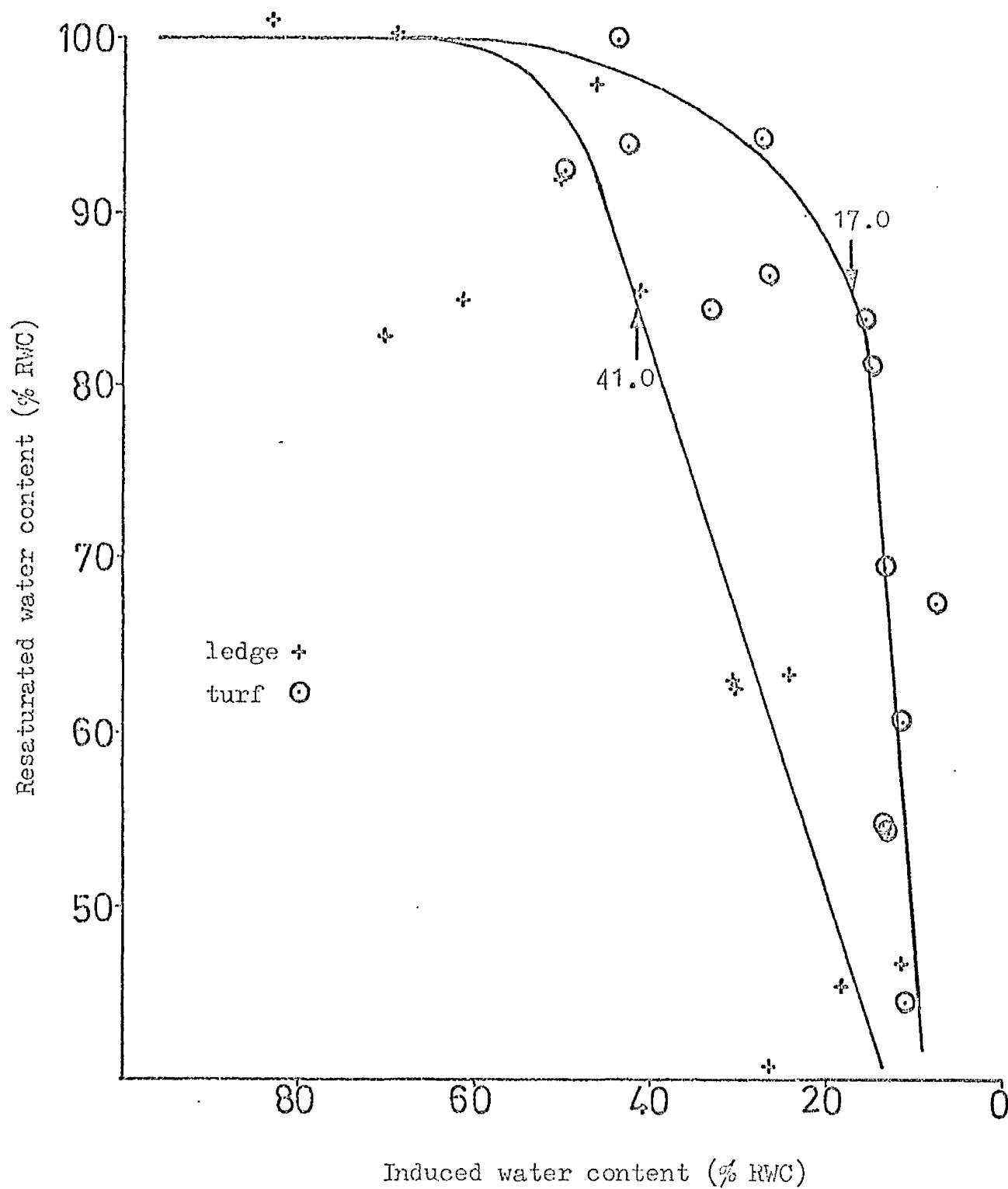
Saturated shoots are placed in an incubator at $28 \pm 0.5^{\circ}\text{C}$ and illuminated with a 100 watt bulb. Loss in weight is followed at half hourly intervals for three hours, by which time the stomata have closed. Weighings are made on an automatic balance. Dry weights are then obtained by oven drying the shoots at 105°C for 3-5 hours.

The RWC is calculated for each weighing (e.g. Weatherley, 1950), and a graph is drawn of RWC against time (e.g. Bannister, 1964), (Figure 19a). Each point is the mean of four replicates. The rate of loss per quarter of an hour is measured from this graph and plotted in Figure 19b. By extrapolating the initial rate of loss (stomatal phase) and the final rate of loss (cuticular phase), the time of stomatal closure is determined. By relating this time back to the first graph of RWC/time, a critical value for stomatal closure is determined as a relative water content (Figure 19a). Variations in stomatal closure point have been followed throughout the year for all species.

Measurement of drought hardness

A standard droughting procedure has been used. Fourteen weighed, saturated shoots are placed in an incubator at $28 \pm 0.5^{\circ}\text{C}$ and illuminated with a 100 watt bulb. In pairs, they are removed after 1, 2, 3, 5, 7, 9 and 11 hour exposures, weighed, and replaced in saturation chambers for 24 hours at room temperature. After this period, the shoots are removed, blotted dry, reweighed, and subjectively assessed for damage. Dry weights are then obtained by oven drying at 105°C for 3-5 hours. Relative water contents are calculated at each stage, and the graph

Figure 20. Resaturation curves and determination of 85%
 Resaturation Points, Vaccinium vitis-idaea,
 Ben Lui, ledge and turf forms, 3-4-69.



plotted of resaturated water content against induced water content (Figure 20) (cf. Rychnovská-Soudková, 1963).. A definite breaking point is observed, below which the shoots do not fully resaturate.

For comparisons between plants it is essential to have a definite critical value which can be used to describe the drought hardness of each sample. From early experiments, an hypothesis was formed that those samples which became resaturated to 85% RWC or greater usually recovered, whereas samples not reaching this point usually showed irreversible damage.

Bannister (1970) has shown that the relationship between resaturation and damage depends upon -

- (1) time of year at which the correlation was made, especially in V. myrtillus which may have a different relationship for leaves and stems,
- (2) time allowed for visual damage to develop, with an increase in damage over longer equilibration times.

In the present study, the drought hardness of V. myrtillus and V. uliginosum has been followed only in the summer when leaves are on the shoots, and the time allowed for visual damage to develop has been 24 hours in all species, no matter what the time of year. There is no evidence to suggest that the resaturation/visual damage relationship in V. vitis-idaea varies with time throughout the year, however, which means that a selected resaturation level is equivalent to the same amount of damage throughout the year.

Regressions of resaturation against subjective estimates of damage

Figure 21. Regression of visual damage on resaturation, Vaccinium myrti

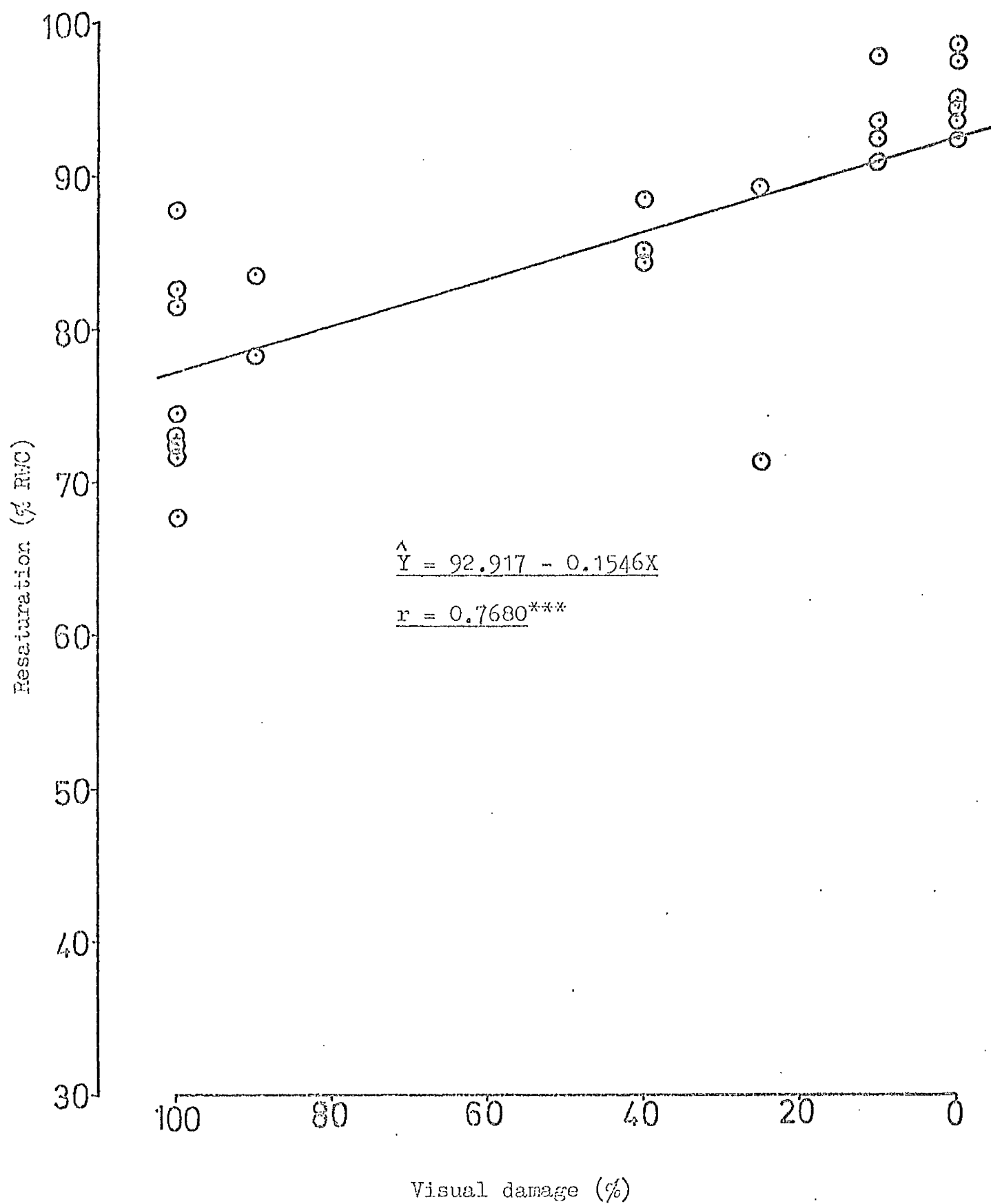
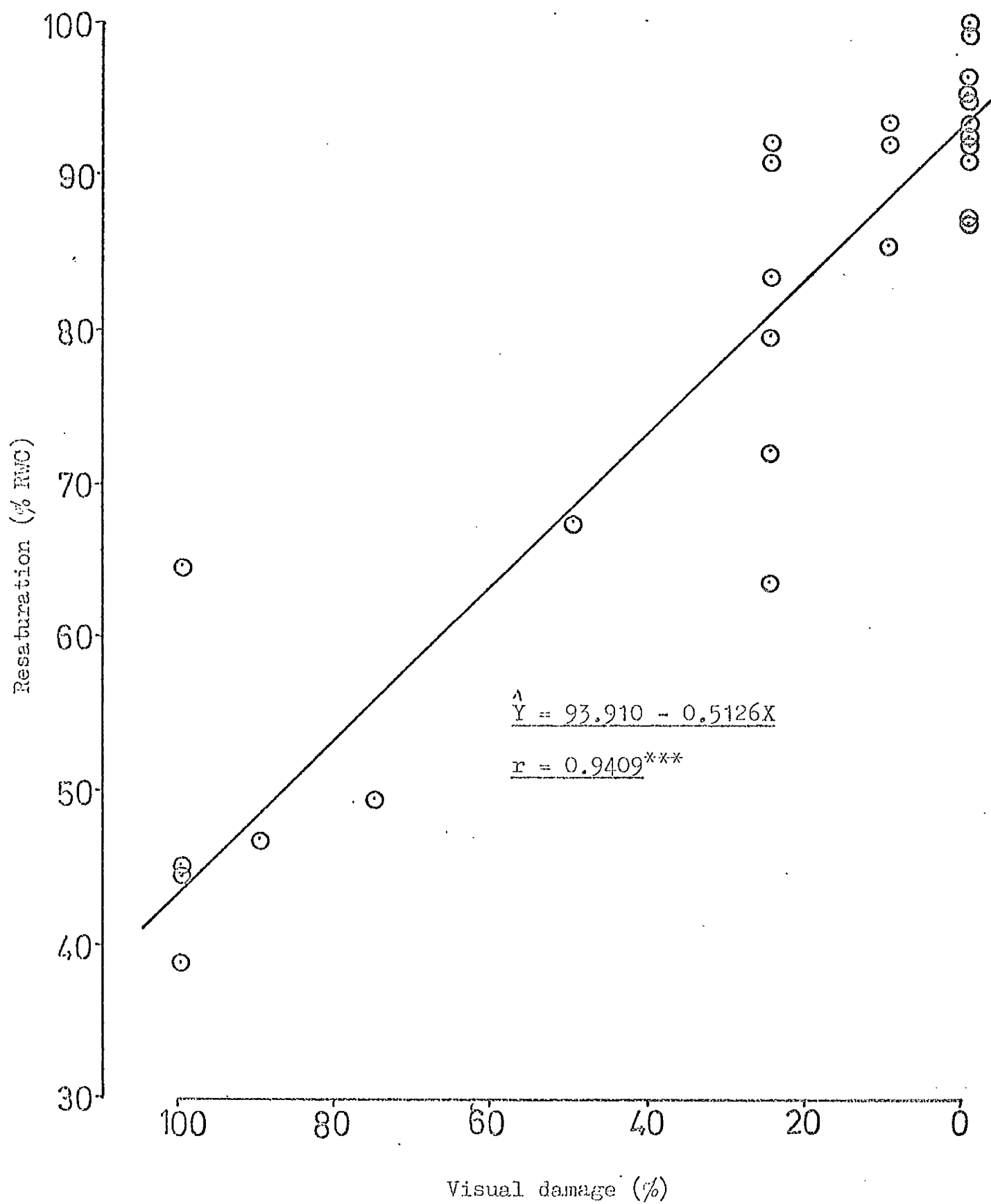


Figure 22. Regression of visual damage on resaturation, Vaccinium vitis-



have been carried out for 28 resaturation values of Vaccinium myrtillus and 32 resaturation values of Vaccinium vitis-idaea in summer, (Figures 21 and 22). In the former, a significant correlation is observed ($r = 0.7680^{***}$, $p < 0.001$), the 85% resaturation point corresponding to an approximate value of 50% damaged material. In the latter, a significant correlation is also observed ($r = 0.9409^{***}$, $p < 0.001$), the 85% resaturation point corresponding to an approximate value of 20% damaged material.

There thus appears to be a different response to droughting by Vaccinium myrtillus as compared to Vaccinium vitis-idaea, with the critical injury point occurring at a higher level of visual damage in the former than in the latter. Vaccinium uliginosum, another deciduous species, appears to be like Vaccinium myrtillus in its relationship between resaturation and visual damage in summer. Oppenheimer (1932, 1963) has used a 20% damage point as a critical level of injury in evergreens, and the 85% resaturation point in Vaccinium vitis-idaea would appear to agree with this 20% critical level. Bannister (1970) has also shown that the 85% level would seem to correlate with a 20% point of damage. From the graph (Figure 20), the induced water content which gives exactly 85% resaturation has been used as the critical injury point of the tissues and can probably be equated with Oppenheimer's (1963) "permanent turgor loss point". In the present study the critical point of injury is thus expressed as the water content of the tissue which gives exactly 85% resaturation and will be designated the "85% resaturation point". Variations in this value have been followed throughout the year for V. vitis-

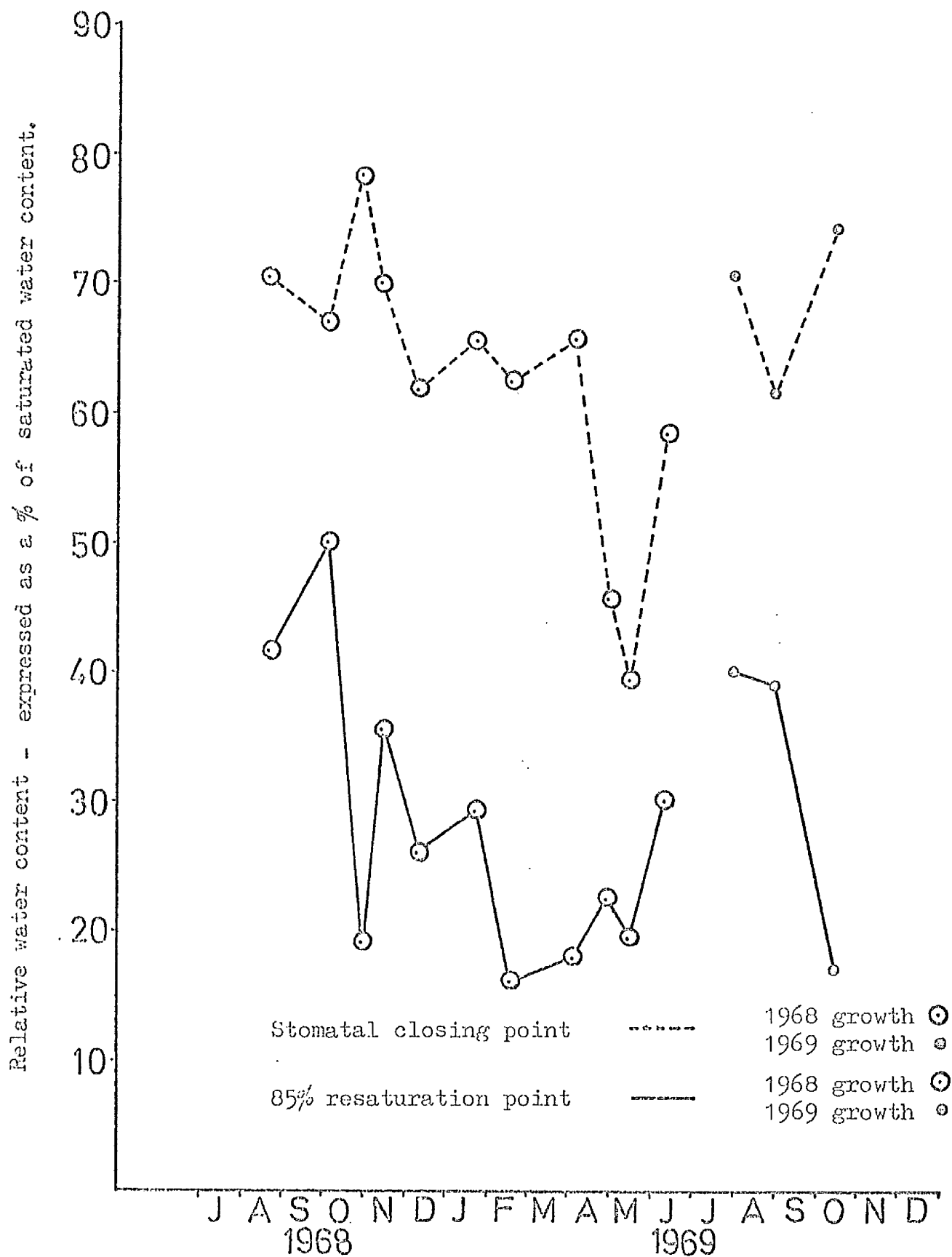


Figure 24. Seasonal variation in drought resistance

Vaccinium vitis-idaea, Ben Lui, turf form.

idaea, and during the summer for V. myrtillus and V. uliginosum.

The percentage moisture content of shoots at saturation (as percentage D.W.) has also been followed for the three Vaccinium species, but only for the material from Ben Lui.

3. Results

Using analysis of variance, the variation throughout the period of study in relative water content at stomatal closure of all Vaccinium vitis-idaea samples (mean of 4 values) has been found to be highly significant ($p < 0.01$), and a difference between any pair of values of about 4.5% relative water content (least significant difference) is considered to be significant. Because the 85% resaturation point consists of a single interpolated value, it has not been possible to carry out an analysis of variance on seasonal variation. However, the amplitude of variation in 85% resaturation values appears to be similar to variation in stomatal closure points.

Figures 23 and 24 show the variation in stomatal closing points and 85% resaturation points of V. vitis-idaea, Ben Lui, ledge and turf forms respectively. An increase in stomatal closing value is indicative of an increase in drought resistance, since early closing means less water loss. A decrease in 85% resaturation point indicates an increase in drought hardness since it means recovery from a lower moisture content.

In both forms, the stomatal closing points are well above the 85% resaturation values, indicating that stomata close in both forms substantially before the point of lethal moisture content is reached. The greatest differences are found from December-May in V. vitis-idaea, ledge form and from October-April in the turf form.

In the ledge form, the stomatal closing points show the highest closing levels from December to April and the lowest level in early May. The turf form, however, shows no peak, but a plateau from December to April with very low closing levels in early May. The plateau occurs during the period of snow-cover whereas the ledge form was uncovered. Although both forms show similar levels of stomatal closing in late summer 1968, the difference between them over the total period of measurement is very highly significant ($p < 0.001$) using analysis of variance.

The decrease in stomatal closing levels in both species in the spring indicates a greater capacity for growth since gas exchange and photosynthesis can then operate over a greater range of stomatal opening. This effect is best seen in the turf form in the early spring before new growth is observed, and it is possible that reserves are being built up fast during this period.

Eighty five percent resaturation points in both forms show a peak in October and another period of high resistance in late winter and early spring. V. vitis-idaea, turf form consistently recovers from smaller induced moisture contents than the ledge form, a difference of at least 15% RWC being observed between them. This indicates a greater drought hardness in the turf form which in February, 1969 recovered from an induced moisture content of 16% RWC. Both forms show most susceptibility in summer and early autumn.

Multiple regression analyses were carried out to relate the four parameters, temperature, daylength, age and rainfall, to variation in both stomatal closing points and 85% resaturation points. Results are presented in Tables 15 and 16.

Table 15. Regression equations and significance of regression. *V. vitis-idaea*,

Ben Lui, ledge and turf forms

Species	Regression equation	% variation due to regression	Variance (F) ratio	Significance of regression
<u>Vvi l</u> , Lui Stomatal closing point	$\hat{Y} = 84.9320$ $-0.3129X_1 - 0.6814X_2$ $+0.0016X_3 - 1.3572X_4$	22.70	0.587	Not significant
85% resaturation point	$\hat{Y} = 39.4244$ $-0.5191X_1 + 1.6212X_2$ $-0.0375X_3 - 7.8757X_4$	43.73	1.554	Not significant
<u>Vvi t</u> , Lui Stomatal closing point	$\hat{Y} = 77.2027$ $+0.1174X_1 - 0.6770X_2$ $-0.0465X_3 + 16.6060X_4$	57.58	2.714	Not significant
85% resaturation point	$\hat{Y} = 35.6578$ $+0.1283X_1 + 0.7561X_2$ $-0.0668X_3 - 12.2689X_4$	44.75	1.620	Not significant

Vvi l, Lui = Vaccinium vitis-idaea, Ben Lui, ledge form

Vvi t, Lui = V. vitis-idaea, Ben Lui, turf form

From Table 15 it is seen that none of the regressions significantly contribute to variation in Y, and percentage variation in Y due to the regression is also small. It would seem that the measured parameters do not have much effect on the drought resistance of the Vaccinium vitis-idaea forms on Ben Lui. However, of the individual parameters (Table 16), age (X_3) appears to contribute a large fraction to both stomatal closing points and 85% resaturation points, with daylength (X_2) being the next most important. In V. vitis-idaea, ledge form, 85% resaturation point, daylength is predominant with age being next in importance.

Table 16. Standard partial regression coefficients (β) and percentage contribution of individual factors, V. vitis-idaea, Ben Lui, ledge and turf forms

	Stomatal closing points				85% resaturation points			
	<u>Vvi l</u> , Lui		<u>Vvi t</u> , Lui		<u>Vvi l</u> , Lui		<u>Vvi t</u> , Lui	
	β	%	β	%	β	%	β	%
X_1	-0.2041	21.3	0.0586	1.0	-0.3316	18.7	0.0543	5.0
X_2	-0.3208	33.5	-0.2451	4.1	0.7489	42.4	0.2321	21.3
X_3	0.4009	41.9	-5.4342	90.0	-0.5131	29.1	-0.6173	56.8
X_4	-0.0312	3.3	0.2823	4.9	-0.1733	9.8	-0.1840	16.9

X_1 = mean temperature

X_3 = age

X_2 = mean daylength

X_4 = mean rainfall

Although the greatest drought hardness appear to occur during the driest period of the year at 2,250 ft. (765 m) on Ben Lui (Figure 2), mean rainfall contributes only a very small amount to variation in 85%

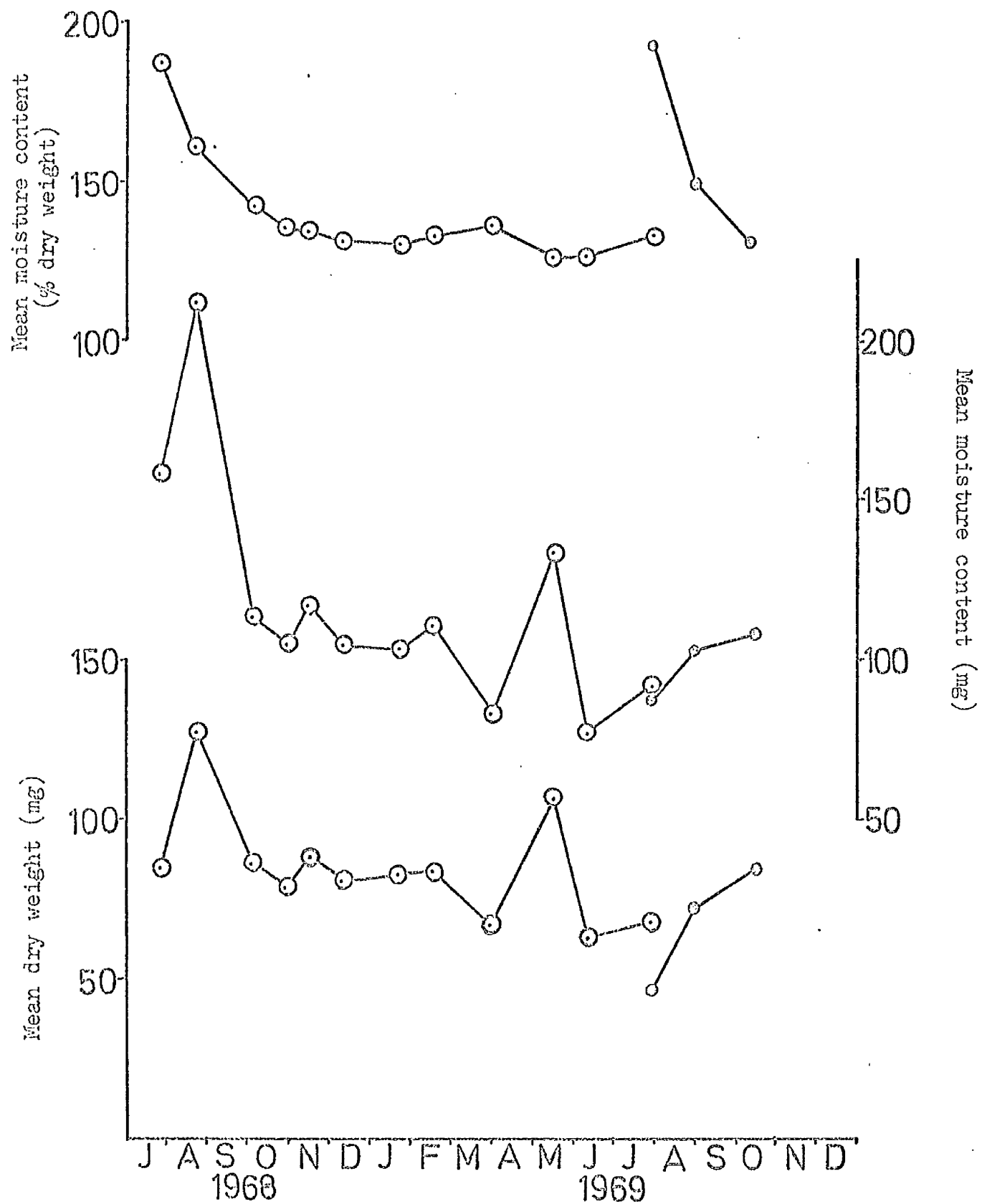


Figure 25. Seasonal variation in saturated moisture content

Vaccinium vitis-idaea, Ben Lui, ledge form

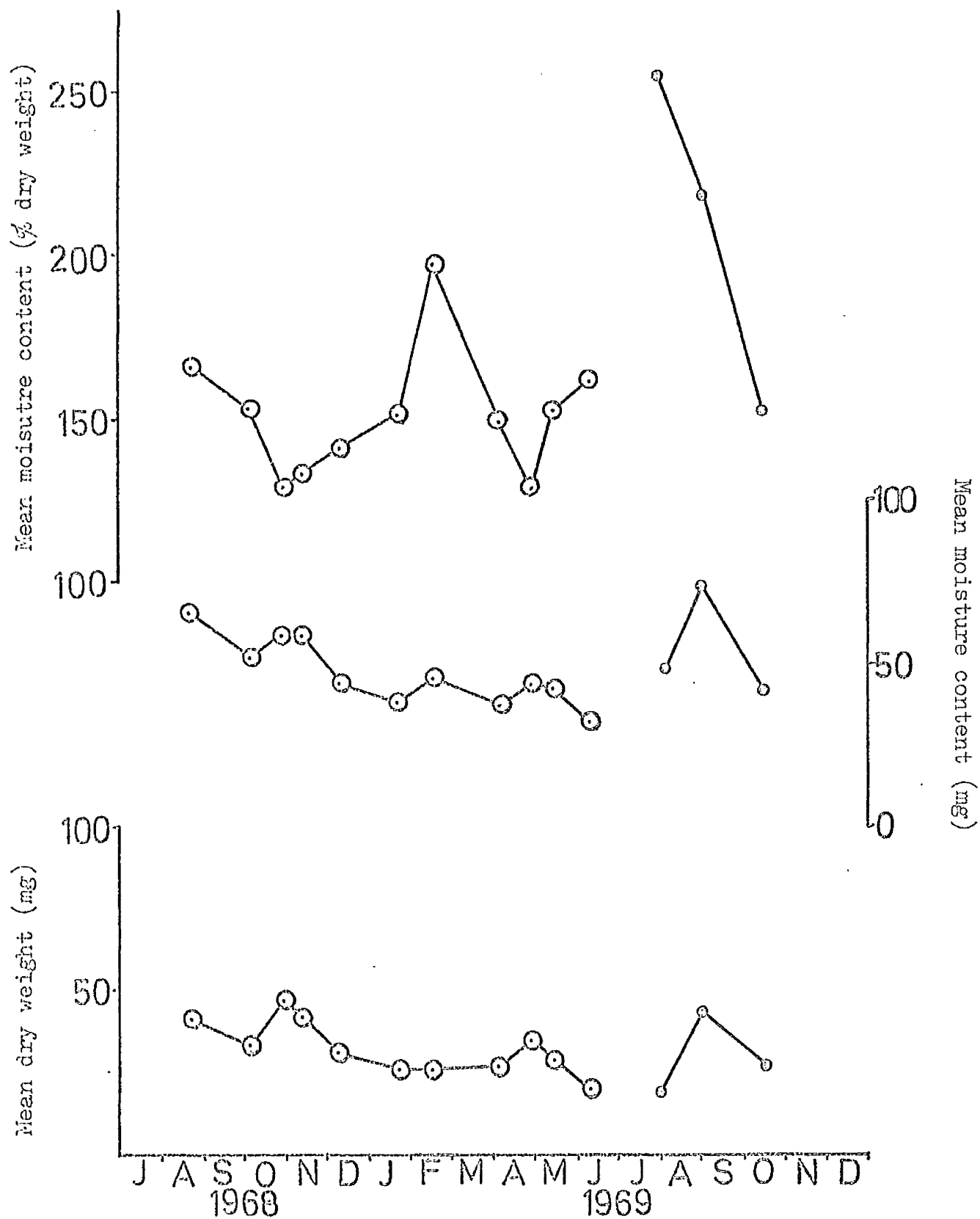


Figure 26. Seasonal variation in saturated moisture content

Vaccinium vitis-idaea, Ben Lui, turf form

resaturation values. It is probable that the excessive rainfall at the high altitude does not allow deficits to develop with resulting hardening.

Transpiration rates, both with open and closed stomata, were consistently found to be greater in V. vitis-idaea, turf than in the ledge form (Figure 19).

Large differences are thus evident between V. vitis-idaea, ledge form and V. vitis-idaea, turf form with respect to their water relations. The higher transpiration and greater drought hardness, coupled with significantly smaller plants of V. vitis-idaea turf form (see Table 1 and section on site descriptions), indicate greater xeromorphic characteristics in this form. Since it has been shown that the turf habitat is more xeric (see site description section), Vaccinium vitis-idaea appears to have adapted to this habitat both by reduction in size of the phenotype and also by physiological adaptations.

The environmental factors measured do not seem to influence drought resistance to the same extent as the frost resistance, though daylength appears to have the greatest influence, but age appears to play the largest part in the regression.

Figures 25 and 26 show the seasonal variation in saturated moisture content of the two forms of V. vitis-idaea, expressed as percentage of dry weight. Total moisture content and total dry weight have also been included to give some indication of how fluctuations in percentage moisture content may occur.

Percentage moisture content of V. vitis-idaea, ledge form is high in summer and falls during winter and spring to its lowest point in June,

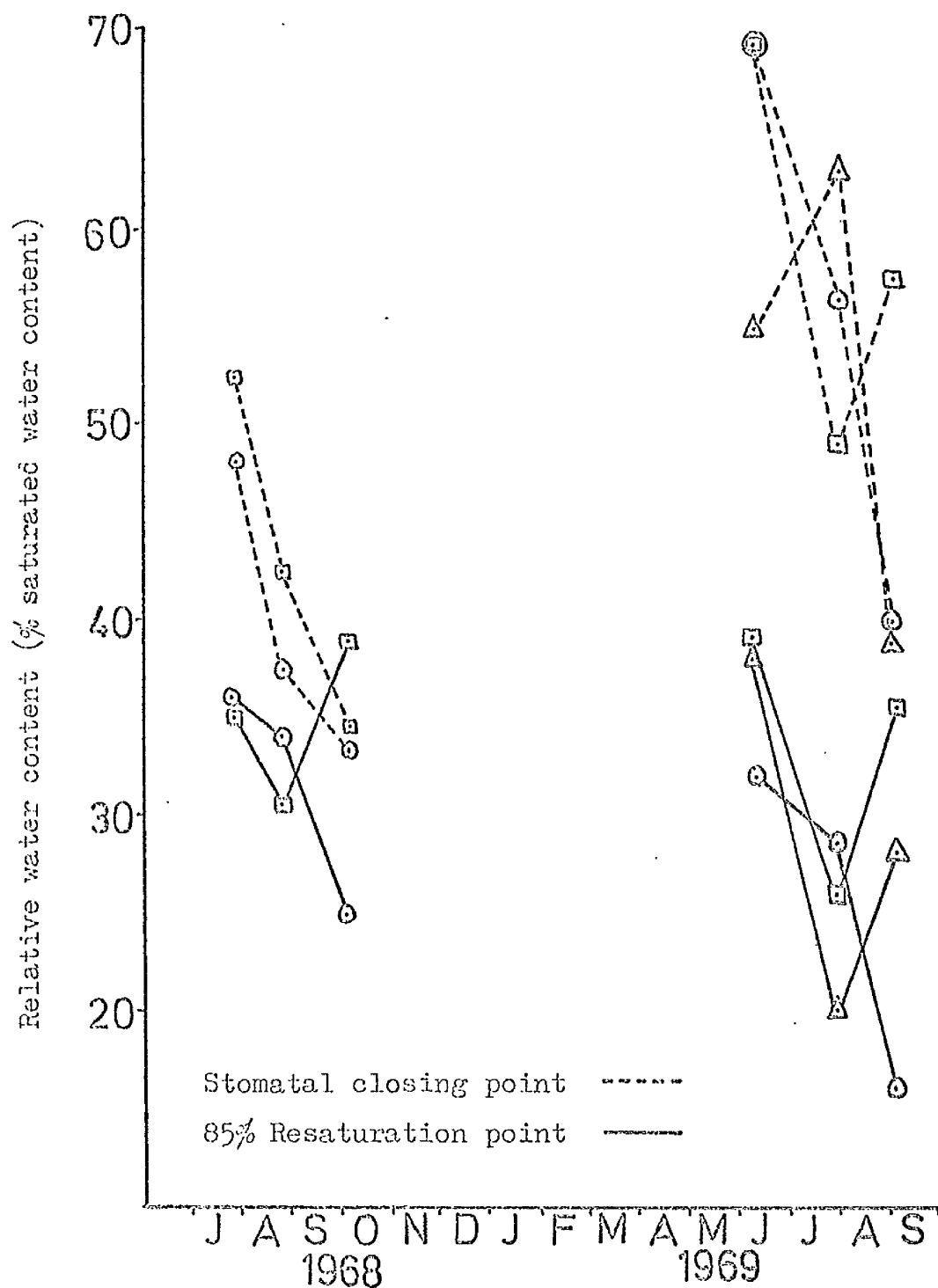


Figure 27. Seasonal variation in drought resistance, Ben Lui.

Vaccinium uliginosum Δ ; *V. myrtillus*, ledge ◻ ;

V. myrtillus, turf ⊙ .

before the new growth which has a very high percentage moisture content due to very small dry weights. The fall in percentage moisture content in new growth is due to dry weight increasing at a faster rate than the actual moisture content, which is also increasing. A similar situation is observed in the turf form, the large increase in February being due to an actual rise in moisture content, the dry weight remaining constant. This indicates that the water absorbing capacity of V. vitis-idaea may be increased under snow early in winter. Havas (1969) has shown that in V. myrtillus, water may be taken in by the aerial shoots from snow or rain.

Bannister (1970) found that drought hardiness in the species he studied was correlated with the reciprocal of percentage moisture content. However, no significant correlation is observed in the present study between percentage moisture content and drought hardiness or stomatal closing points in either form of V. vitis-idaea.

The difference in size between the two forms is emphasised by the consistent large differences in dry weights throughout the year. Dry weights in the ledge form are continuing to rise in October, 1969, perhaps a sign of continuing growth and thus immaturity.

Figure 27 presents the seasonal variation in drought resistance of V. myrtillus, ledge and turf forms and V. uliginosum, Ben Lui.

In general, all samples have high stomatal closing values at the beginning of the season but low values at the end. It would appear that as the leaves age and eventually senesce, stomatal control of transpiration is virtually lost.

In V. myrtillus, ledge form and V. uliginosum, drought hardiness (85%

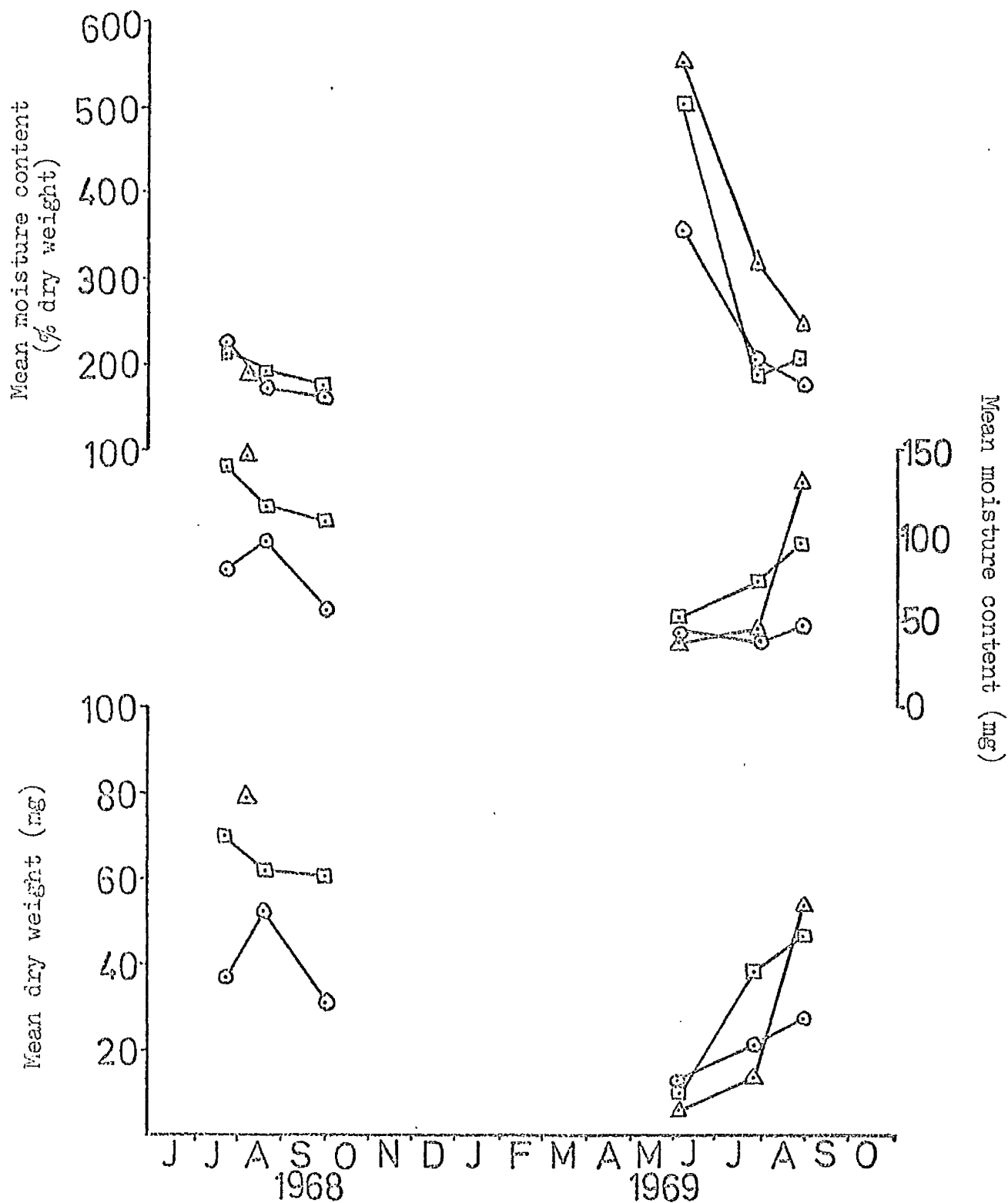


Figure 28. Seasonal variation in saturated moisture content, Ben Lui.

Vaccinium uliginosum Δ ; *V. myrtillus*, ledge ◻ ;
V. myrtillus, turf ○ .

resaturation points) appears to increase in July then decrease again by September, 1969. However, V. myrtillus, turf form, appears to continue to harden throughout both seasons 1968 and 1969.

All stomatal closing points are above the lethal moisture content level except in V. myrtillus, ledge form, when in October, 1968, stomatal closing values were up to 6% RWC lower than the 85% resaturation points. This means that in October, 1968, V. myrtillus, ledge form, was very susceptible to droughting. This situation has not occurred in 1969.

V. myrtillus, ledge and turf forms, seem to have similar hardness early in the season, but as the plants become more mature, the differences between them become obvious, the ledge form being the more susceptible. V. uliginosum behaves like V. myrtillus, ledge form.

The seasonal variations in saturated moisture content of the three deciduous samples on Ben Lui are given in Figure 28.

Percentage moisture contents appear to parallel the stages of growth, decreasing rapidly as the increase in dry weights is greater than the increase in total moisture contents. Although growth appears to be continuing in September, 1969, it appears to have stopped by that time in 1968. The senescence of the leaves is probably responsible for this, and unfortunately no information is available for stems alone.

V. uliginosum appears to have a higher moisture content than the two forms of V. myrtillus. The forms of V. vitis-idaea (Figures 25 and 26) appear to have a slightly lower moisture content than V. myrtillus, especially in very young material.

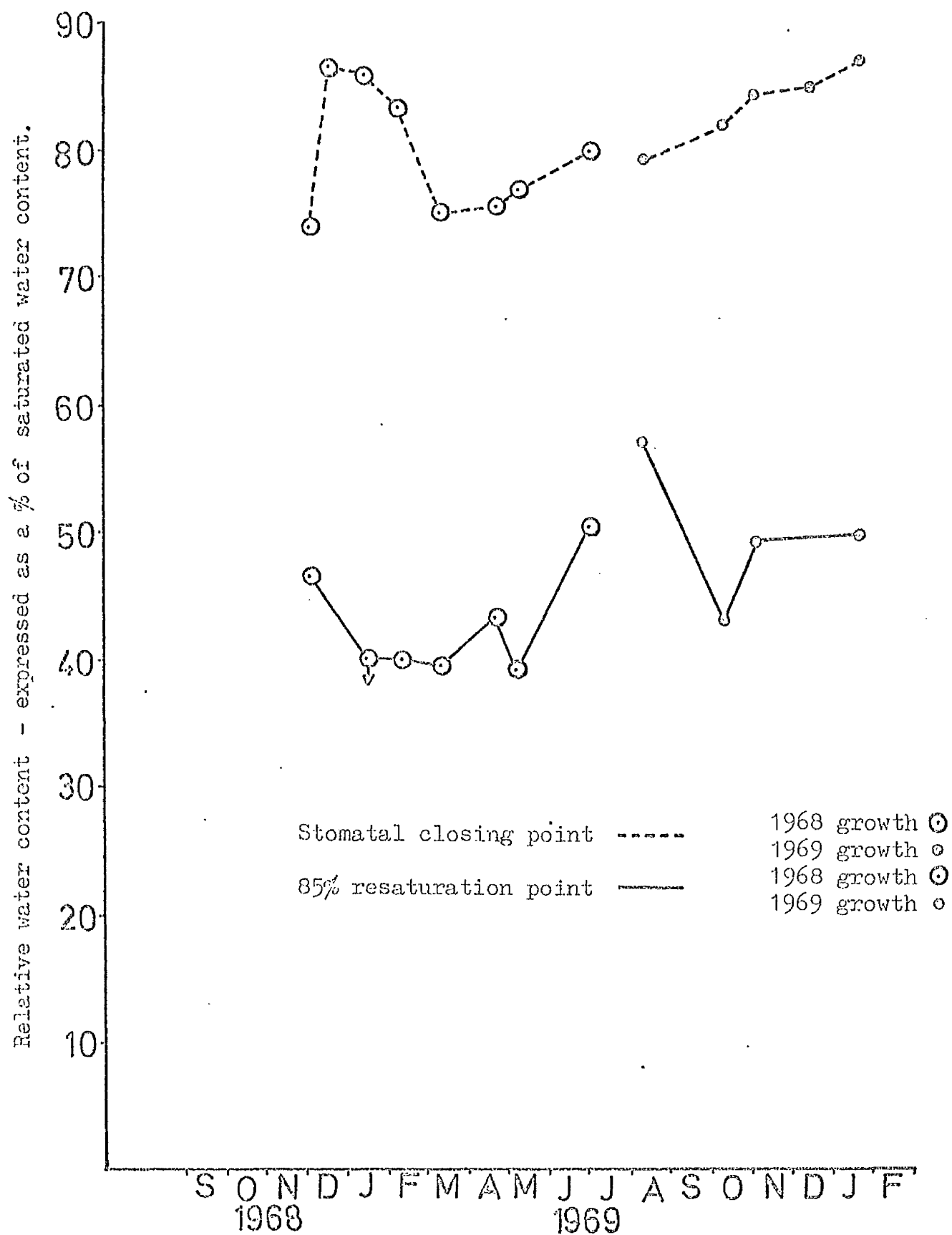


Figure 29. Seasonal variation in drought resistance

Vaccinium vitis-idaea, Loch Katrine.

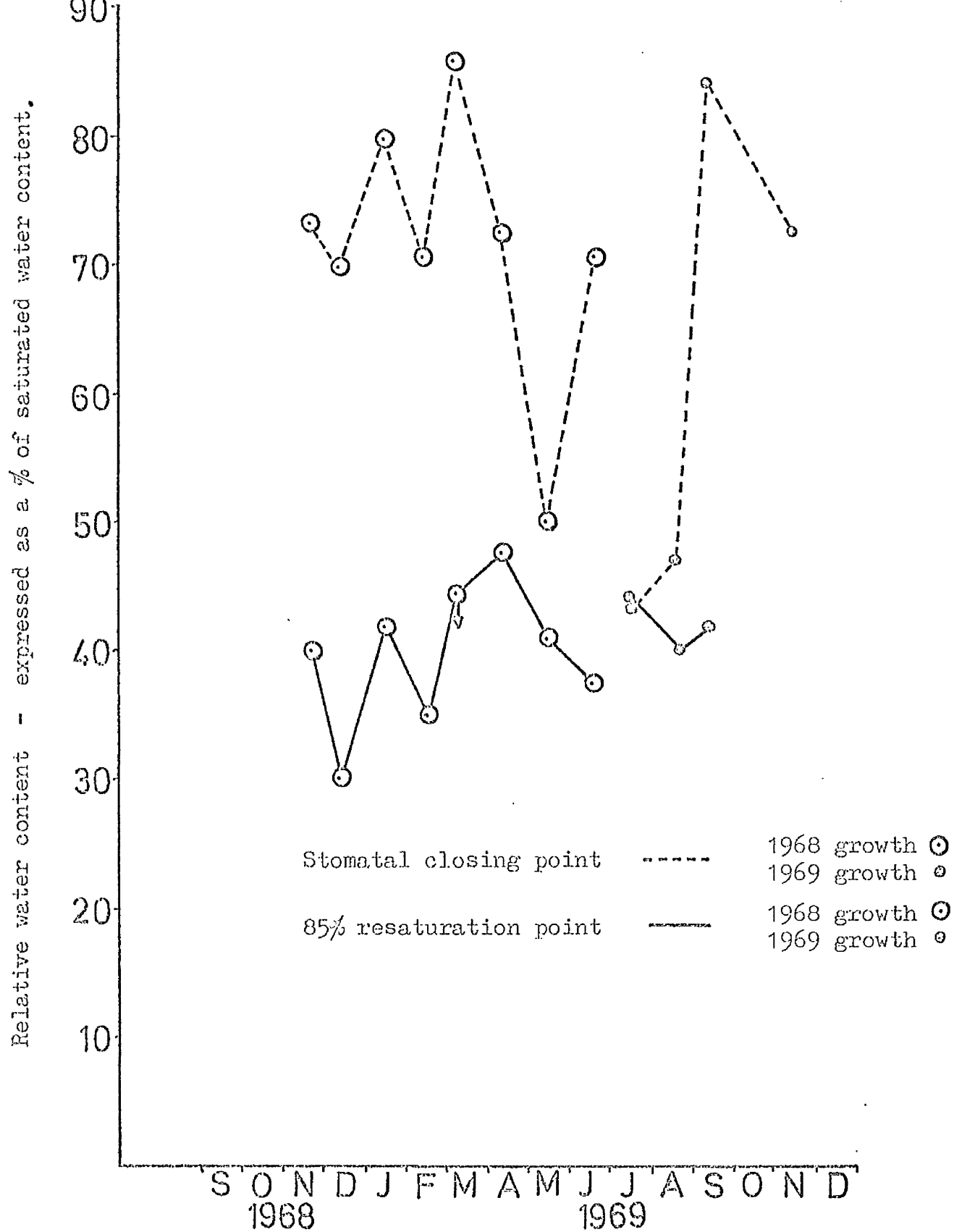


Figure 30. Seasonal variation in drought resistance

Vaccinium vitis-idaea, Malham Tarn.

V. myrtillus from the two lowland sites of Milngavie Moor and Garelochhead shows little variation in either stomatal closing points or 85% resaturation points. Values are shown in Table 17.

Table 17. Stomatal closing points and 85% resaturation points
(in RWC) of V. myrtillus, Milngavie Moor and
Garelochhead, 1969

Date	Milngavie Moor		Garelochhead	
	Stomatal closing point	85% resaturation point	Stomatal closing point	85% resaturation point
5. 5.69	-	-	59.0	40.5
2. 7.69	55.5	33.5	42.5	34.0
11. 8.69	54.0	33.5	47.0	30.5
7.10.69	64.5	30.5	64.0	26.0
3.11.69	62.0	30.0	-	-

Stomatal closing points are always greater than lethal moisture contents in both samples. Although of a similar drought hardness over the season, V. myrtillus, Garelochhead, appears to show poor stomatal control of transpiration compared with Milngavie Moor. The greater shading in the former, affecting the growth and development of the plant, is thought to induce loss of stomatal control.

Figures 29 and 30 show the variation in drought resistance of V. vitis-idaea, Loch Katrine and Malham Tarn respectively.

Stomatal closing levels are high in winter and low in spring, as in the forms on Ben Lui. The pattern of stomatal closing in Loch Katrine material is very similar to that of Ben Lui, ledge form (Figure 23).

V. vitis-idaea, Malham Tarn, shows a large drop in May which is within 7.5% RWC of the 85% resaturation level on the same date. The young material of this sample shows very poor stomatal control, the stomatal closing point being below the 85% resaturation point in July and very close to it in August. Maturation, however, causes a large improvement in stomatal control. Although Loch Katrine material does not show low stomatal values in May, material from Malham Tarn appears to forgo a degree of stomatal control in order to increase the time of assimilation in the spring, as in the Ben Lui forms.

Eighty five percent resaturation points in V. vitis-idaea, Loch Katrine, appear similar to those of V. vitis-idaea, Ben Lui, ledge form, being more drought hardy in winter and spring, with the young material being the most susceptible. Malham Tarn material shows irregular values of hardness though peaks are seen in December and February.

Multiple regression analyses were carried out and the results are presented in Tables 18 and 19.

Table 18 shows that none of the regressions contribute significantly to variation in Y, the percentage variation due to regression being very small in all cases except the 85% resaturation point of V. vitis-idaea, Loch Katrine. It is thought that the small number of readings is causing this non-significance. Apart from this sample it would seem that the measured parameters again have little effect on the variation in drought resistance.

Table 18. Regression equations and significance of regression. V. vitis-idaea,

Loch Katrine and Malham Tarn

Species	Regression equation	% variation due to regression	Variance (F) ratio	Significance of regression
<u>Vvi Kat</u> Stomatal closing points	$\hat{Y} = 90.9212$ $+1.2675X_1 - 2.0541X_2$ $+0.0315X_3 - 20.4719X_4$	37.24	0.742	Not significant
85% re-saturation points	$\hat{Y} = 33.4077$ $+ 0.0647X_1 + 1.0991X_2$ $- 0.0278X_3 + 28.2561X_4$	80.11	5.033	Not significant (small number of readings)
<u>Vvi Malm</u> Stomatal closing points	$\hat{Y} = 93.2873$ $+0.7998X_1 - 3.3936X_2$ $+0.0592X_3 - 23.8688X_4$	47.59	1.362	Not significant
85% re-saturation points	$\hat{Y} = 32.9956$ $-0.0886X_1 + 0.5576X_2$ $-0.0084X_3 + 34.5007X_4$	34.79	0.800	Not significant

Vvi Kat = Vaccinium vitis-idaea, Loch Katrine

Vvi Malm = V. vitis-idaea, Malham Tarn

Table 19. Standard partial regression coefficients (β) and percentage contribution of individual factors

	Stomatal closing points				85% resaturation points			
	<u>Vvi Kat</u>		<u>Vvi Malm</u>		<u>Vvi Kat</u>		<u>Vvi Malm</u>	
	β	%	β	%	β	%	β	%
X_1	1.6382	35.2	0.3603	21.3	0.0603	3.5	-0.1213	10.2
X_2	-1.7542	37.7	-0.7908	46.7	0.6737	39.4	0.4317	36.4
X_3	0.7476	16.1	0.4450	26.4	-0.4651	27.3	-0.1845	15.6
X_4	-0.5118	11.0	-0.0955	5.6	0.5087	29.8	0.4485	37.8

X_1 = mean temperature

X_3 = age

X_2 = mean daylength

X_4 = mean rainfall

Table 19 indicates once again that daylength (X_2) is the predominant factor in all samples, though similar to rainfall (X_4) in V. vitis-idaea, Malham Tarn, 85% resaturation point. Rainfall is the next important influence in V. vitis-idaea, Loch Katrine, 85% resaturation point, with age also being important. Age does not appear as important as in the two Ben Lui forms of V. vitis-idaea. Temperature (X_1) appears to contribute to regression in V. vitis-idaea, Loch Katrine, stomatal closing points. It would appear that in contrast with the forms on Ben Lui, the lower rainfall at Loch Katrine and Malham Tarn allows water deficits to occur and thus cause an increase in drought hardiness.

Discussion and conclusions

It is obvious from the results, that the important conclusions do not arise from the multiple regression analysis as in the frost resistance section, but from comparisons between different sites and different species.

Bannister (1964) has shown that Calluna from drier sites has lower stomatal closing levels than Calluna on wet or moist sites. He suggested that Calluna is adapted to drier habitats not by greater transpirational control but by an ability to forgo a degree of stomatal protection for the sake of continued assimilation.

In the present study, differences between Vaccinium vitis-idaea, ledge and turf forms are attributable to the adaptation of the turf form to the more xeric habitat. This latter form exhibits greater transpiration, lower stomatal closing points and greater hardiness, as well as morphological differences such as smaller shoot and leaves, a greater number of stomata per unit area and smaller stomata than the ledge form (Table 1). Morphological differences between V. vitis-idaea, ledge form and V. vitis-idaea, Loch Katrine, do not appear to be reflected in their physiology, stomatal closing points and 85% resaturation points being very similar. However, rates of water loss do appear to be less in the Loch Katrine sample. The main differences thus appear to be between the different morphological forms of V. vitis-idaea, the large leafed forms, i.e. Ben Lui, ledge, Loch Katrine and Malham Tarn being similar, with the small leafed turf form exhibiting a greater xeromorphism. These differences are thought to reflect the moisture conditions of the habitat, the turf habitat being the most xeric.

On Ben Lui, these differences are also seen in V. myrtillus but only when the plants have become more mature. Young plants exhibit a similar drought resistance.

Of the lowland types, V. myrtillus growing in woodland appears to

have poorer stomatal control of water loss compared with the sample on the open moor, perhaps because the former is growing in conditions of higher, less fluctuating relative humidities and therefore exhibits more hygrophytic characteristics. Hygen (1953a) has shown that the more hygrophytic samples appear to have a poor stomatal closing reaction to water loss.

Differences between species in the present study appear to be between the large leafed forms of the xeromorphic species V. vitis-idaea, and the rest, no distinction being made between V. myrtillus and V. uliginosum. The best stomatal control is seen in the large leafed xeromorphic group whereas the stomata of all other samples close at a much lower relative water content.

Although having little significance, the environmental factor mainly influencing the drought resistance of V. vitis-idaea forms is daylength, with age also playing a large part. The older the plant, the greater is its drought hardiness.

Bannister (1970) has found correlations of daylength with the drought hardiness of Erica cinerea and Erica tetralix when the proximity to the nearest equinox was used. He found that hardiness was at a minimum about these points, increasing as the days lengthened or shortened, and reaching peaks around the winter and summer solstices. He found also that age played a part in the variation of drought hardiness in the two Erica species.

From the graphs of variation in drought hardiness of V. vitis-idaea, no equivalent relationship with the equinoxes appears to exist. It is thought, however, that the relationship of drought hardiness with the

environment is complex, and this probably contributes to the non-significance of the factors considered.

V. vitis-idaea and V. myrtillus thus appear to have adapted successfully to the different ecological habitats under different moisture regimes. It is thought that the moisture factor is not important in their distribution, at least within the range of moisture regimes observed.

(c) The relationship between frost and drought resistance

Frost and drought resistance have often been found to be correlated, and a review of the literature is given by Levitt (1956).

In an early work, Pisek and Larcher (1954) were able to show that the needles of Pinus cembra and Picea abies from the timberline, and leaves of dwarf Ericaceous shrubs, exhibited a yearly cycle of drought resistance, the greatest resistance being found in winter and the least in summer. The patterns they found generally paralleled the yearly cycle in frost hardness of the same species as measured by Ulmer (1937) and Pisek and Schiessl (1947). Their comparison was one of amplitude rather than being specific, frost and drought resistance both having maxima in the winter and minima in the summer.

Larcher (1963) discusses the problem in evergreens, and gives a model which delimits two types -

- (a) showing no correlation between frost and drought hardness,
and
- (b) showing strong correlation between frost and drought
hardness.

The first type, growing where the occurrence of frost is limited to only three months in winter, exhibits a slight degree of frost hardening in winter and no increase in drought hardness. This type is very susceptible to damage caused by ice formation in its tissues ("Eisempfindlich"). The other type, growing where frosts occur in more than six months of the year, becomes very frost hardy in winter and shows a general correlation with drought hardness. This type is very resistant to the formation of

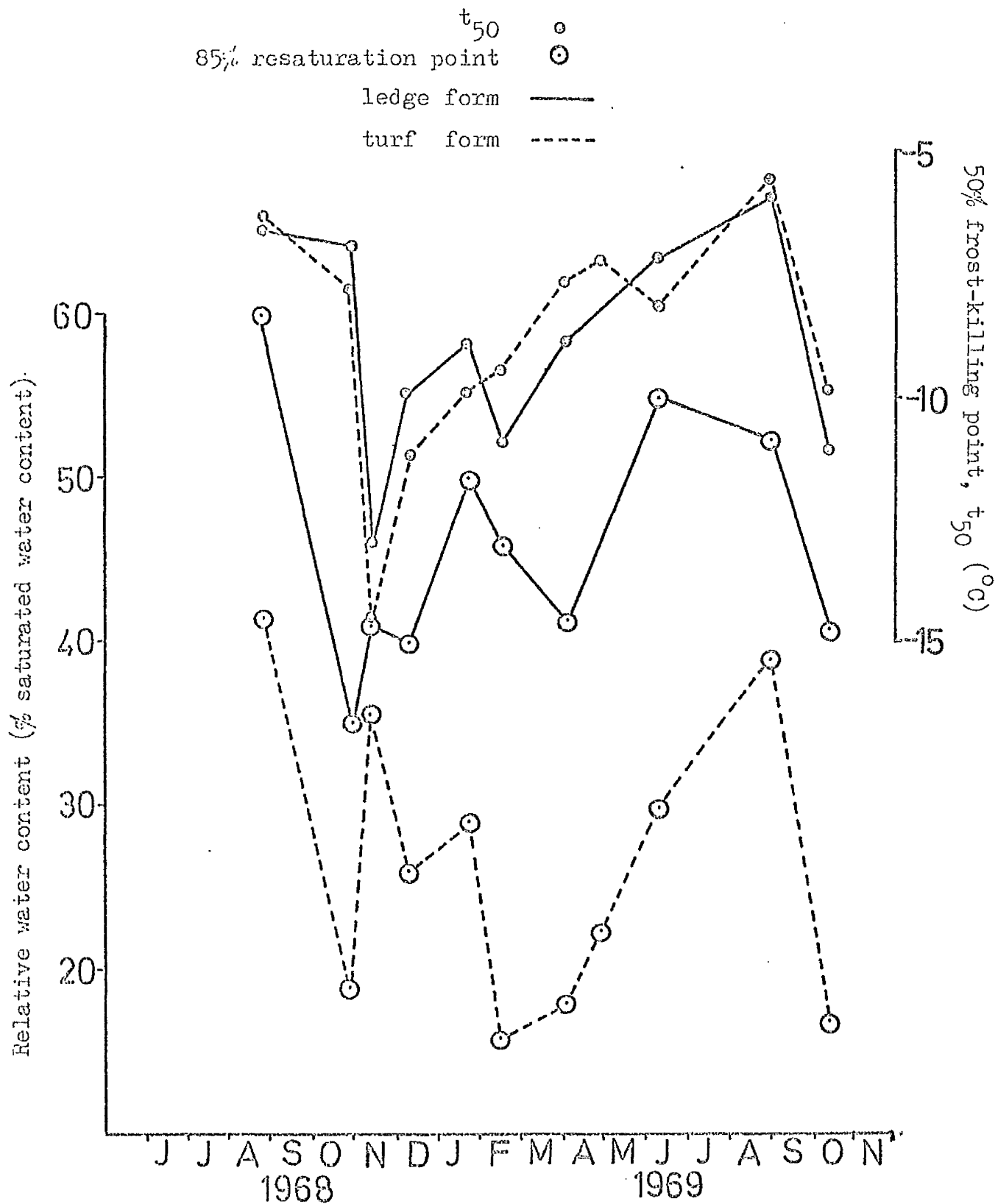


Figure 31. Frost and drought hardiness, Vaccinium vitis-idaea,
Ben Lui, ledge and turf forms.

ice in its tissues ("Eisbeständig"). Larcher (1963) concludes, therefore, that a correlation between drought and frost resistance is therefore only to be expected if the frost hardness is "real" frost hardness, i.e. the endurance of ice formation.

Kappen (1964) has studied the frost, drought and heat resistance of ferns, and has found that the seasonal curves of frost and drought resistance generally run parallel, both having maxima in winter and minima in summer, although no specific correlations were made.

It is evident from his work with poikilohydric ferns in particular, that drought resistance has a much broader curve than frost resistance, greater drought resistance developing earlier in autumn, and remaining high in the spring while the frost-susceptibility increases.

Similar results have been found in the present study. Figure 31 shows the frost and drought hardness curves for Vaccinium vitis-idaea, Ben Lui, ledge and turf forms. It is seen that although the frost and drought maxima are in winter, and minima are in summer, the drought hardness of both forms of V. vitis-idaea has the much broader curve.

Unlike most of the previous work on this problem, specific pairs of frost and drought hardness values have been taken for the period of this study and correlation coefficients have been calculated to establish the significance of the correlation between frost and drought hardness.

As well as ordinary correlation coefficients (r), rank correlation coefficients ($r_s = 1 - \frac{6 \sum d^2}{n(n^2-1)}$) have also been calculated (see Snedecor and Cochran 1967, p.193). This latter calculation does not take the magnitude of the changes into account, but emphasises the direction of

these changes. The results for four forms of V. vitis-idaea are presented in Table 20.

Table 20. Ordinary correlation coefficients (r) and rank correlation coefficients (r_s) between t_{50} values and 85% resaturation points

Species	r	r_s
<u>Vvi l</u> , Lui.	+ 0.5000	+ 0.4909
<u>Vvi t</u> , Lui	+ 0.0860	+ 0.2636
<u>Vvi Kat</u>	- 0.0833	+ 0.0699
<u>Vvi Malm</u>	+ 0.1083	+ 0.1000

(all values are non-significant)

Vvi l, Lui = Vaccinium vitis-idaea, Ben Lui, ledge form.

Vvi t, Lui = V. vitis-idaea, Ben Lui, turf form.

Vvi Kat = V. vitis-idaea, Loch Katrine.

Vvi Malm = V. vitis-idaea, Malham Tarn.

No significant correlation is observed, although the rank correlation has slightly improved the value for V. vitis-idaea, Ben Lui, turf form and caused V. vitis-idaea, Loch Katrine to become positive.

An explanation for the non-significant correlations was sought first by examining Larcher's (1963) two types of evergreen. However, V. vitis-idaea appears to fit into the "Eisbeständig" type, which does show a general correlation.

The more likely explanation is seen by examination of Figure 31 where it is evident that the increase of drought hardiness in autumn is more marked than the increase in frost hardiness, and more particularly in the

spring the frost susceptibility increases while the drought hardiness remains high. This appears to indicate a differential response of frost and drought hardiness to the environment. This is substantiated by the fact that the environmental factors studied have been found to contribute significantly to the frost hardiness of most species (see frost resistance section), whereas no significant contributions have been found towards drought hardiness (see drought resistance section).

From the evidence presented in this study it is not possible to explain the differential response to the environment, and particularly to explain why frost susceptibility increases in the spring while drought hardiness remains high. Reasons for this would be purely speculative.

It is obvious, therefore, that a complex situation exists when considering the relationship between frost and drought hardiness, and much more detailed and thorough work is needed to clarify the situation.

However, previous evidence has pointed strongly in favour of a positive correlation between frost and drought hardiness, and the present study has found that, in general, the frost and drought hardiness of V. vitis-idaea also show a positive correlation, although no statistical significance can be attached to the results.

PART III - GROWTH CABINET STUDIES

(a) Introduction

It is well established by controlled environment studies that frost hardiness is induced by factors that reduce plant growth, especially temperatures near or below 0°C and short photoperiods (e.g. McGuire and Flint, 1962; Kohn and Levitt, 1965; Li et al., 1966; Huystee et al., 1967; Irving and Lanphear, 1967a, b; Tumanov, 1967; Steponkus and Lanphear, 1968, van den Driessche, 1969).

It has been indicated that early development in frost hardiness is partly due to the onset of dormancy (i.e. the inability to produce normal growth even under favourable conditions) in the woody plant in late summer and early autumn (e.g. Chandler, 1954). Because of this close association between the onset of dormancy and development of frost hardiness, there evolved an hypothesis that dormancy was necessary for the development of frost hardiness (e.g. Chandler, 1954; van der Veen and Meijer, 1959). Irving and Lanphear (1967a) have shown, however, that the increase of frost hardiness in Acer negundo and Viburnum plicatum tomentosum can be induced under controlled conditions, with long days and certain low temperatures, without the prerequisite of dormancy. Increasing duration of exposure to short days followed by a low temperature hardening period in darkness, brought about a progressive increase in hardiness. They found that the short day stimulus could be reversed by long days. Thus they have shown that development of hardiness is a

photoperiod response. McGuire and Flint (1962), Huystee et al. (1967) and van den Driessche (1969) have all shown that photoperiod has a marked effect on the frost hardening of plants. van den Driessche (1969) has concluded that the factors important in development of frost hardiness in Douglas-fir seedlings are daylength, nighttemperature and light intensity.

On the other hand, the dormant condition appears to be important in maintaining frost hardiness. Irving and Lanphear (1967c) have shown that the dormant condition helped to maintain the hardy conditions of Acer and Viburnum when the plants were exposed to 70°F. Application of dormin to non-dormant plants also prevented dehardening during warm temperature exposure. van den Driessche (1969) has concluded that photoperiod has no influence on loss of hardiness in Douglas-fir, temperature being the only factor effective in causing dehardening. Linking this latter conclusion with that of Irving and Lanphear (1967c), it would seem that plants will deharden only if in the non-dormant state, and that this loss of hardiness is often influenced by temperature.

In the present study, two experiments under controlled conditions have been carried out:

- (1) to determine the relative effects of temperature and photoperiod on bud-break and subsequent growth of the three Vaccinium species in their winter condition,
- (2) to determine the relative effect of temperature and photoperiod on the development of frost hardiness in the three Vaccinium species in their summer condition.

(b) MaterialsBud-break and growth experiment

Material was collected in November, 1967 and potted. Vaccinium myrtillus was obtained from Milngavie Moor [26/553763, 350 ft. (106 m)] and Uplawmoor [26/439557, 475 ft. (144 m)], a woodland habitat. V. vitis-idaea was obtained from Loch Katrine [27/496075, 450 ft. (153 m)] and V. uliginosum was collected from Ben Arthur, Argyllshire [27/263062, 2,300 ft. (762 m)] and Beinn Narnain, Argyllshire [27/267066, 2,300 ft. (762 m)].

Frost hardening experiment

V. myrtillus and V. vitis-idaea, ledge forms, were collected in April, 1969 from Beinn an Lochainn, Argyllshire [27/222073, 1,600 ft. (486 m)] and placed in wooden boxes. Pots of V. uliginosum were used from the previous collection.

(c) Methods(i) Growth Cabinets

Five growth environment cabinets have been used for Experiment I and three cabinets for Experiment II.

The growth cabinets consist of two types:-

- (1) This type is divided into two separate compartments in which day-length can be altered independently while maintaining the same temperature conditions throughout the whole cabinet. Temperature fluctuations of $\pm 1^{\circ}\text{C}$ were recorded at the temperatures used. The interior walls of this cabinet are lined with metalised Melinex and only slight

fluctuations in light intensity can be recorded in a horizontal plane. The plant trays are fixed 4 ft. 6 ins. below the lamps which consist of 5 ft. long, 65W fluorescent tubes 3 inches apart, alternating one white or daylight tube and one warm white tube. Supplementary illumination is obtained from two 5 ft., 65W fluorescent tubes.

This type of cabinet was built in Glasgow and is situated at Garscube Research Laboratory.

(2) The second type, built by Saxton (Sax-Air) limited, was used only in Experiment I. It consists of a single growing cabinet and has the facility of allowing selection of different day and night temperatures. Temperature control of $\pm 1^{\circ}\text{C}$ was recorded throughout the experiment. No supplementary light for extending daylength with low intensity light is possible. Illumination is supplied by a double bank of fluorescent tubes and the intensity of light is higher than in the other type of cabinet. Although the walls are lined with metalised Melinex, light intensity is found to be highest in the centre of the cabinets.

Hampton (1967) has described these cabinets in more detail, the ones used in the present study being equivalent to his B and D types.

(ii) Layout

I. The influence of temperature and photoperiod on bud-break and subsequent growth.

Four pots from the frame outdoors, each of Vaccinium myrtillus, V. vitis-idaea and V. uliginosum were placed at random in each of the five following treatments on 29th January, 1969.

A : 8°C , 8 hr. daylength, light intensity 100 ft. candles (1,076 lux),

Cabinet type 2.

B : 5°C, 12 hr. daylength, light intensity 1,000 ft. candles (10,760 lux), Cabinet type 1.

C : 5°C, 18 hr. daylength, light intensity 1,000 ft. candles (10,760 lux), Cabinet type 1.

D : 20°C, 8 hr. daylength, light intensity 1,000 ft. candles (10,760 lux), Cabinet type 1.

E : 20°C, 16 hr. daylength, light intensity 1,500 ft. candles (16,140 lux) Cabinet type 2.

Light intensity was measured at plant height in the centre of the cabinet with an EEL photometer.

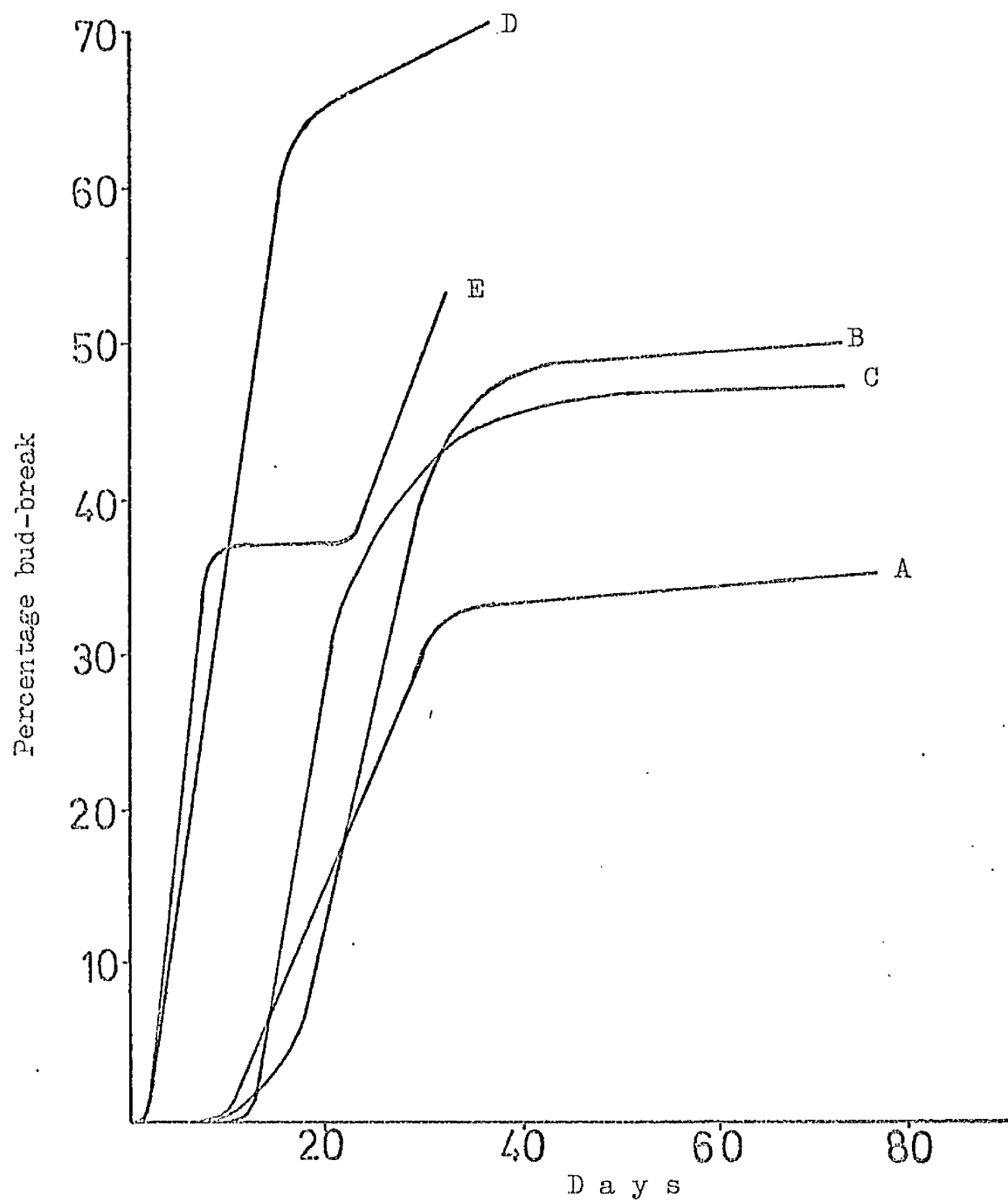
Regular measurements were made of the bud-break, elongation of new shoots and the number of expanded new leaves. Bud-break is expressed as percentage of the total number of buds on the previous year's growth, shoot elongation is expressed as a percentage of the total previous shoot length and the number of newly expanded leaves is expressed as a percentage of the final number of leaves. All treatments were watered regularly throughout the experiment, treatments D and E requiring more watering than A, B and C.

II. Influence of temperature and photoperiod on the development of frost hardiness.

One box from the frame outdoors each of V. myrtillus and V. vitis-idaea, and three pots of V. uliginosum were placed at random in each of the following treatments on 24th June, 1969. All plants were mature by this time.

I : Controls in the frame were subject to natural temperatures and daylengths over the experimental period (see Figures 3 and 5).

Figure 32. Bud-break with time, V. uliginosum



- A : 8°C, 8 hr. light
B : 5°C, 12 hr. light
C : 5°C, 18 hr. light
D : 20°C, 8 hr. light
E : 20°C, 16 hr. light

- II : 18°C, 6 hours daylength + 2 hours supplementary light, light intensity 1,000 ft. candles (10,760 lux) and 100 ft. candles (1,076 lux) respectively.
- III : 5°C, 8 hours daylength + 2 four-hour supplementary light periods before and after full light period, light intensity as in II.
- IV : 5°C, 6 hours daylength + 2 hours supplementary light, light intensity 700 ft. candles (7,532 lux) and 40 ft. candles (430 lux) respectively.

Measurements of frost hardiness in V. myrtillus and V. vitis-idaea were taken at regular intervals, and at the end of the experiment, the number of green leaves, number of yellow leaves still on plant, and the number of leaves dropped were measured for V. myrtillus and V. uliginosum as this gives some indication of the onset of dormancy. No frost hardiness measurements were taken of V. uliginosum since insufficient material was available.

(d) Results

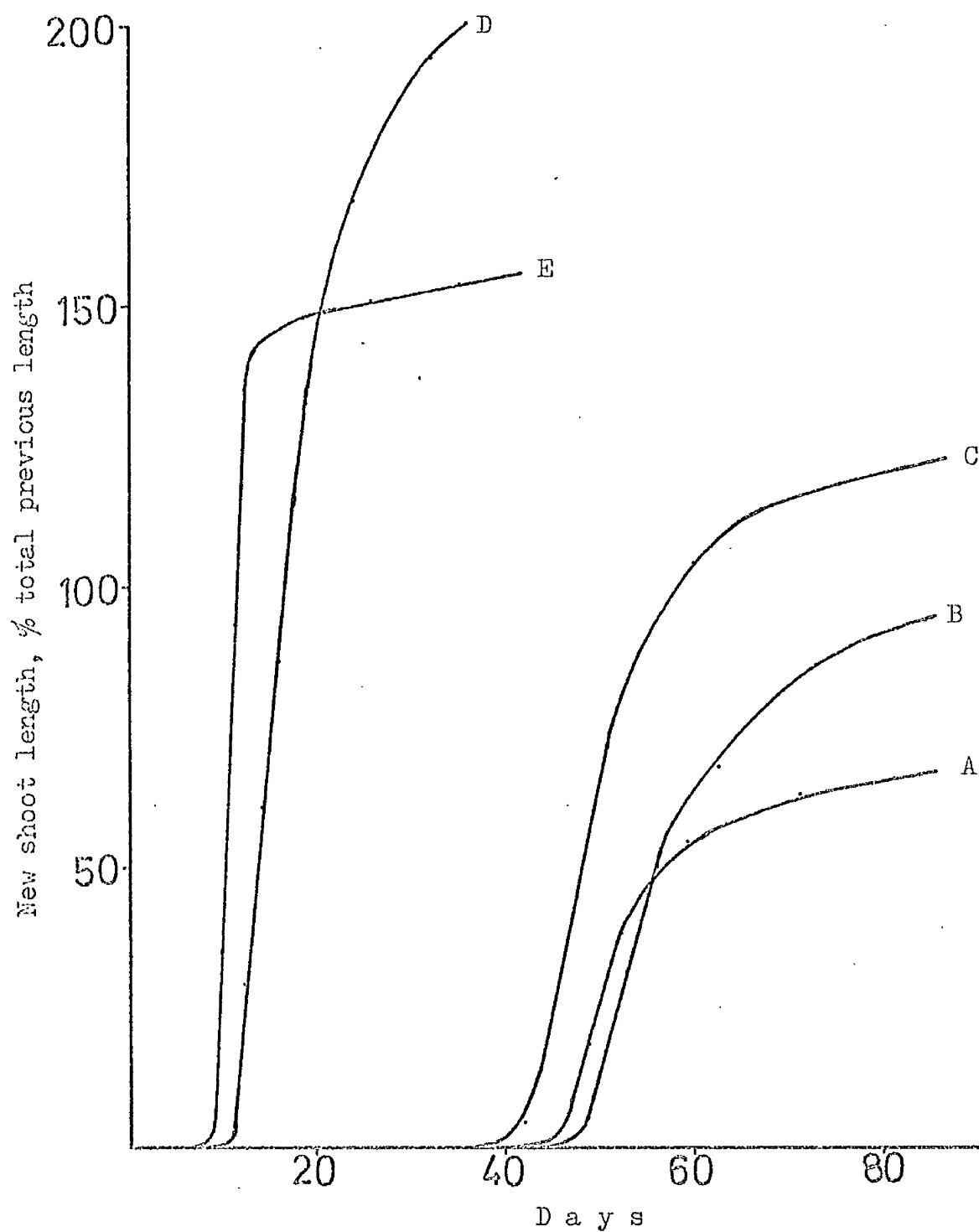
Bud-break and growth experiment

Vaccinium uliginosum

Bud-break at each treatment is given in Figure 32. Each point on the graph represents the mean of four values, except for treatment A in which the mean is of two values.

In treatments A, B and C the first appearance of bud-break after 14-17 days compares with 7 days for treatments D and E. No difference is seen by inspection within these two groups which correspond to low and high temperatures respectively. There is a significant difference between

Figure 33. New shoot length, V. uliginosum



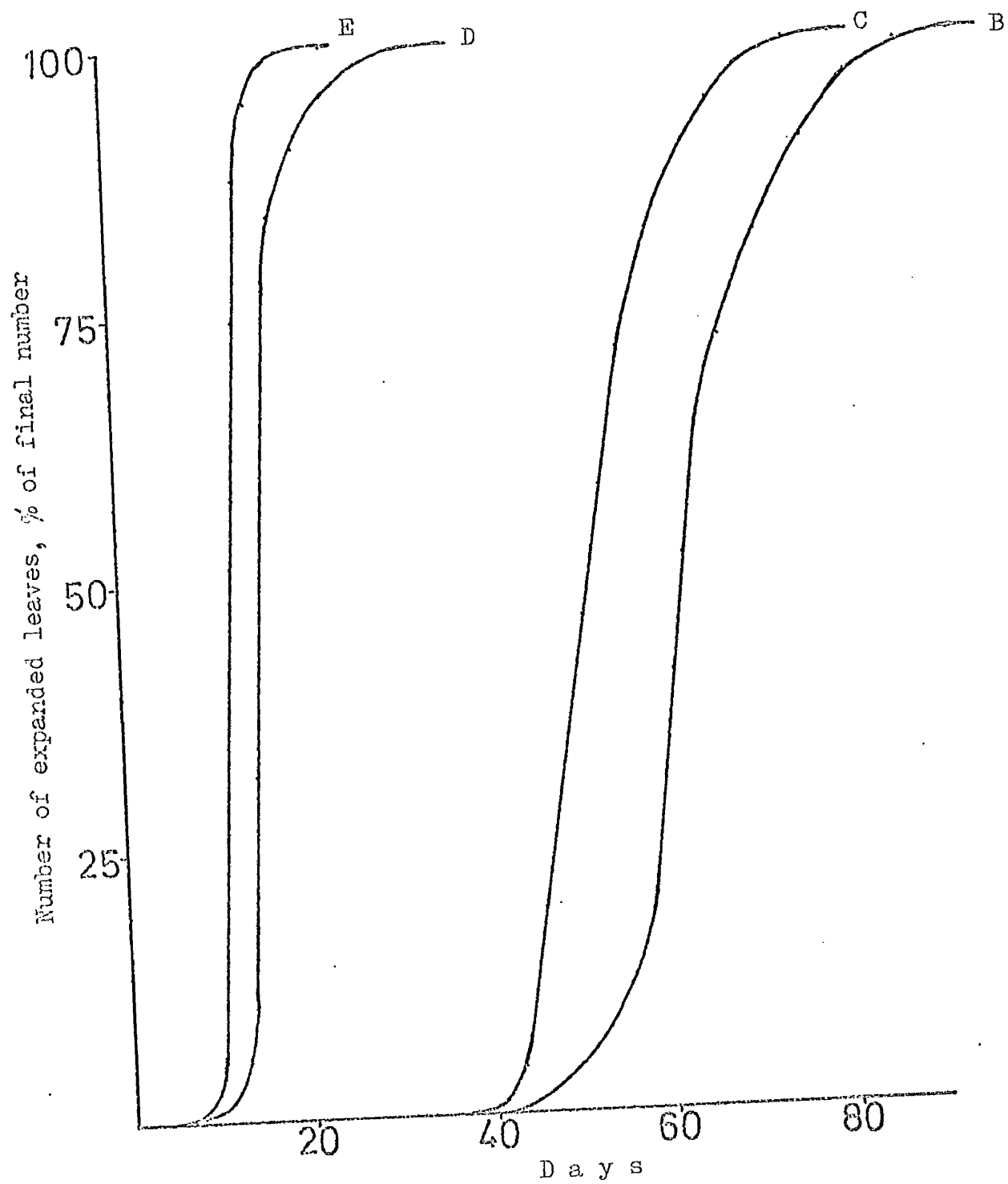
- A : 8°C, 8 hr. light
B : 5°C, 12 hr. light
C : 5°C, 18 hr. light
D : 20°C, 8 hr. light
E : 20°C, 16 hr. light

the times of bud-break for these two groups, $t = 7.19^{***}$ ($p < 0.001$).

The maximum final bud-break appears to be in treatment D, although those in E, after remaining static for 10 days appear to continue to break bud after this period.

The new shoot length for V. uliginosum is given in Figure 33. The time lapse between the two groups at different temperatures, measured by the time to measurable shoot growth, has increased to 30 days. There appears to be a small difference in rate of shoot growth in treatments D and E, with the greatest rate in E, but the maximum length being reached more quickly in the latter treatment. In the group at the lower temperature, the shoots in C appear to have the greatest rate of growth, and those in B and A being similar to begin with but shoots in B increasing over A after 60 days. The final growth values of new shoots in A, B and C have been compared by analysis of variance and results are presented in Table 21, and the least significant difference between the means has been calculated. $F = 9.581^*$ indicates a significant difference at the 5% level of significance ($p < 0.05$). Two degrees of freedom have been lost from the total of 11 because only two replicates were used in A, thus making necessary an estimate of the other two. The main difference between means appears to be between treatments A and C, other differences being non-significant. This non-significance is probably partly due to the very small number of degrees of freedom.

Figure 34. Number of expanded leaves, *V. uliginosum*



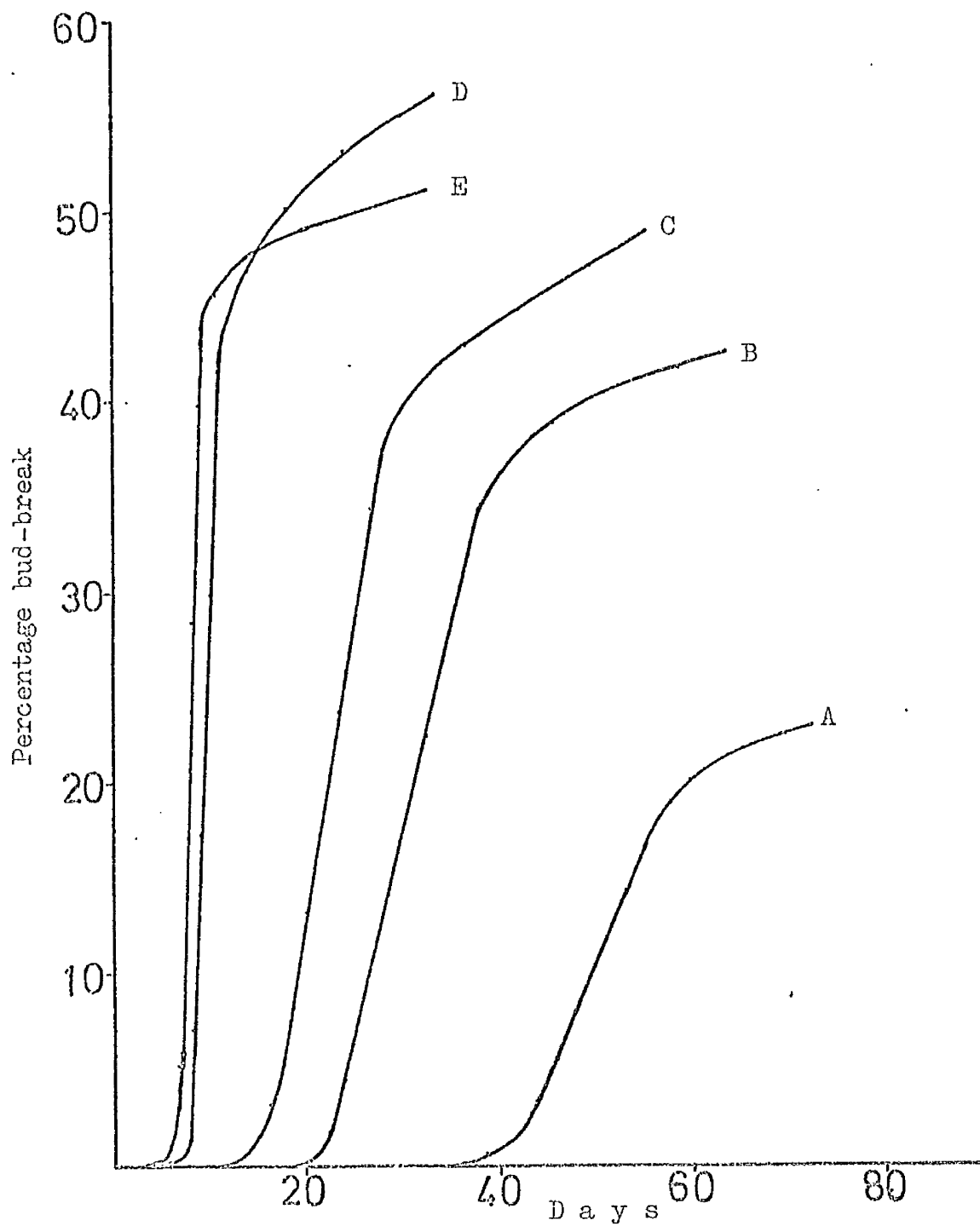
B : 5°C, 12 hr. light

C : 5°C, 18 hr. light

D : 20°C, 8 hr. light

E : 20°C, 16 hr. light

Figure 35. Bud-break with time, V. myrtillus



A : 8°C, 8 hr. light

B : 5°C, 12 hr. light

C : 5°C, 18 hr. light

D : 20°C, 8 hr. light

E : 20°C, 16 hr. light

Ecological aspects of the resistance of plants
to environmental factors

Summary of Ph.D. Thesis presented to Glasgow University by A. Polwart, B.Sc.

This study involved an investigation of the seasonal variation in frost and drought resistance of three Vaccinium species with the aim of determining the influence of environmental factors on the local and geographical distribution of the species.

The study involved -

- A. Monthly samples brought into the laboratory from the field for the determination of -
 - (i) frost resistance by subjection to a series of freezing treatments and the establishment of a 50% killing point.
 - (ii) drought resistance by subjection to various drought stresses and the establishment of (a) stomatal closure points, (b) their ability to recover from a certain water deficit.
- B. Limited growth cabinet studies have been used to investigate the influence of temperature and daylength on the plants.
- C. The relative contribution of temperature, daylength and rainfall to the seasonal variation in the frost and drought resistance of the three species of Vaccinium has been assessed by the use of multiple regression analyses.

From the results the following conclusions have been reached.

1. Daylength is the most important environmental factor influencing the seasonal variation in frost and drought hardiness of the Vaccinium species with the exception of the frost hardiness of the strictly alpine V. uliginosum which is predominantly influenced by temperature.
2. Vaccinium myrtillus is thought to have a longer growing season at more southern latitudes because of the longer winter daylengths causing early dehardening and preventing full frost hardening. This is supported by measurement of bud-break and measurement of induction of frost hardiness under controlled environmental conditions. This species thus may be more susceptible to early or late frosts at more southern latitudes.
3. Upper altitudinal limits of V. myrtillus and V. vitis-idaea are thought to be controlled by low levels of frost hardiness in winter caused by immaturity due to the short growing season at the higher altitudes. These plants are thus susceptible to frost damage at certain times of the year and it is thought that the insulation of snow cover plays an important role in their survival at higher altitudes.

4. V. uliginosum develops a large degree of hardiness in winter and appear to be well adapted to the short season at high altitudes. However, it appears to grow only on moist rock ledges above 2,000 ft. (608 m) on Be Lui, Argyll, because it may not be able to compete for water in the more xeric turf habitat.
5. Low rainfall appears to induce an increase in frost hardiness in lowland species of V. myrtillus and V. vitis-idaea, but has little effect on the drought hardiness. No effect is seen at higher altitudes because of excessive rainfall.
6. V. myrtillus and V. vitis-idaea appear to have adapted to the more xeric turf habitat both morphologically and physiologically. The smaller turf forms exhibit higher transpiration rates, lower relative water contents at stomatal closure and a greater drought hardiness.
7. Frost and drought hardiness of V. vitis-idaea both have maxima in winter and minima in summer. However, no correlation is found when specific values are taken because drought hardiness appears to develop earlier in autumn than frost hardiness, and remains high in spring while the susceptibility to frost increases.

Table 21. Analysis of variance between final growth values in
treatments A, B and C - *Vaccinium uliginosum*

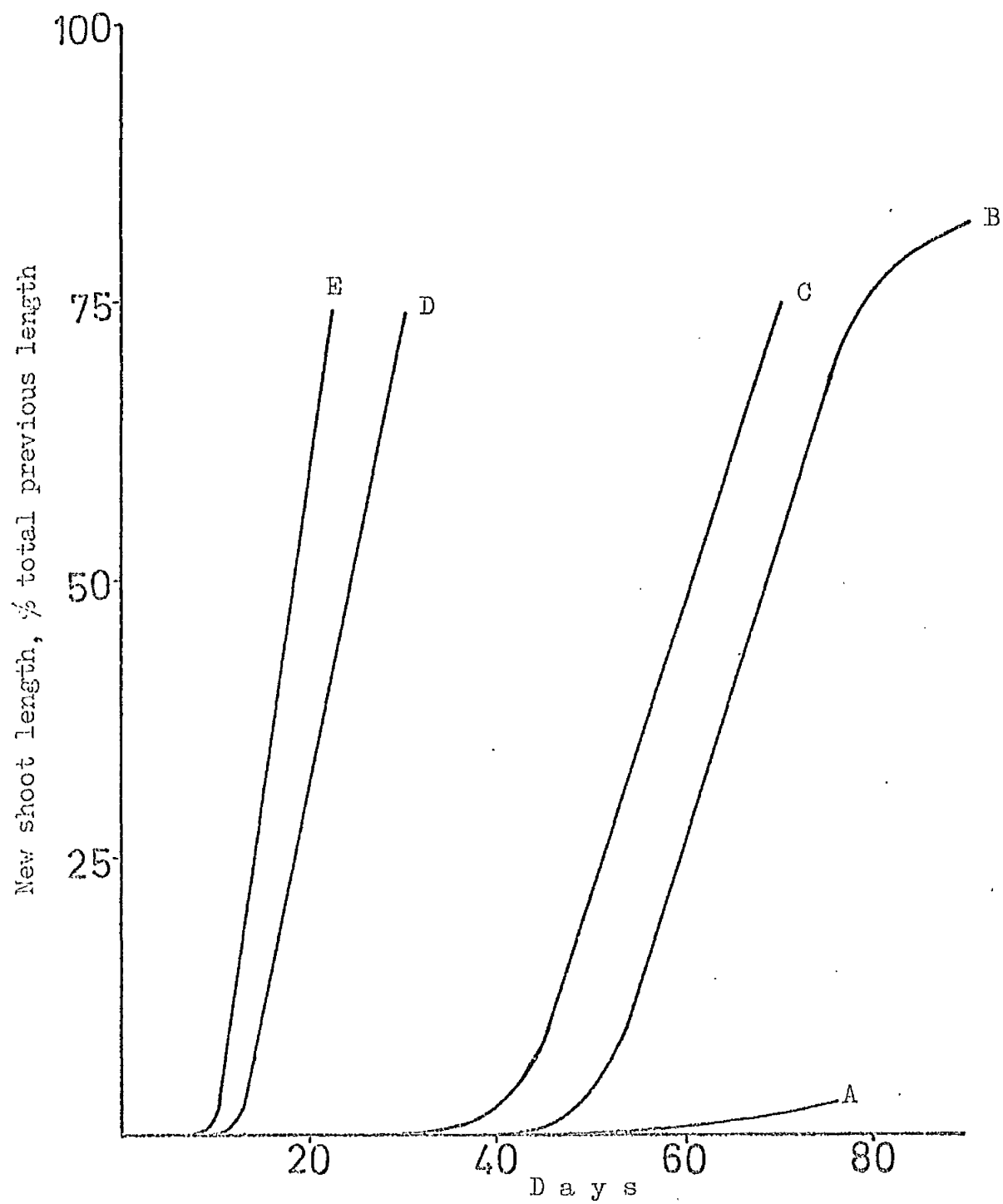
Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	Variance Ratio (F)
Replicates	3	1214.38	404.79	1.409
Treatments	2	5503.57	2751.79	9.581 ^x
Residual	4	1148.87	287.22	
Total	9 (11-2)	7866.82		
Treatment	A	B	C	Least significant difference
Mean	63.09	91.07	115.51	33.31

The number of expanded leaves in *V. uliginosum* is shown in Figure 34. Treatment A has been omitted since the maximum number of expanded leaves had not been attained in the duration of the experiment. The graphs show a reflection of those in Figure 33, with little difference between D and E and the lag in response at treatment B compared with treatment C. This figure will not be repeated for the other two species of *Vaccinium* since, apart from adding little to the information already given by the other two measurements, the shoots at treatments A, B and C, i.e. the low temperature, have not matured in the duration of the experiment and their leaves are not fully expanded.

Vaccinium myrtillus

Bud-break at each treatment is given in Figure 35. The two temperature groups are not so obvious by inspection in this species, so an analysis of variance was carried out between the time to first appearance

Figure 36. New shoot length, V. myrtillus



A : 8°C, 8 hr. light
B : 5°C, 12 hr. light
C : 5°C, 18 hr. light
D : 20°C, 8 hr. light
E : 20°C, 16 hr. light

of bud-break in treatments A to E. Results are given in Table 22. A very highly significant difference is shown by $F = 100.97^{***}$ ($p < 0.001$) and the least significant difference between the means is 4.93 days.

Table 22. Analysis of variance between time to appearance of bud-break in treatments A to E - *V. myrtillus*

Source of Variation	Degrees of Freedom		Sum of Squares	Mean Square		Variance Ratio (F)
Replicates	3		52.95	17.65		1.72
Treatments	4		4098.75	1024.69		100.97***
Residual	12		122.85	10.237		
Total	19		4274.55			
Treatment	A	B	C	D	E	Least significant difference
Mean (days)	47.25	26.00	20.50	10.00	7.00	4.93

Significant differences are seen between the means of the high temperature treatments (D and E) and the means of the low temperature treatments (A, B and C). The means of A, B and C also differ significantly from each other. This indicates significance between the two different temperature groups, and also significance between different light regimes at the low temperature.

The difference between the two temperature groups is very evident in Figure 36. The rate of shoot growth in E is much greater than that in D. Although the shoots in C appear to have the same rate of growth as in B, the length at any particular time is greater in C because of the earlier bud-break at that treatment. Shoots in A show very little growth at all.

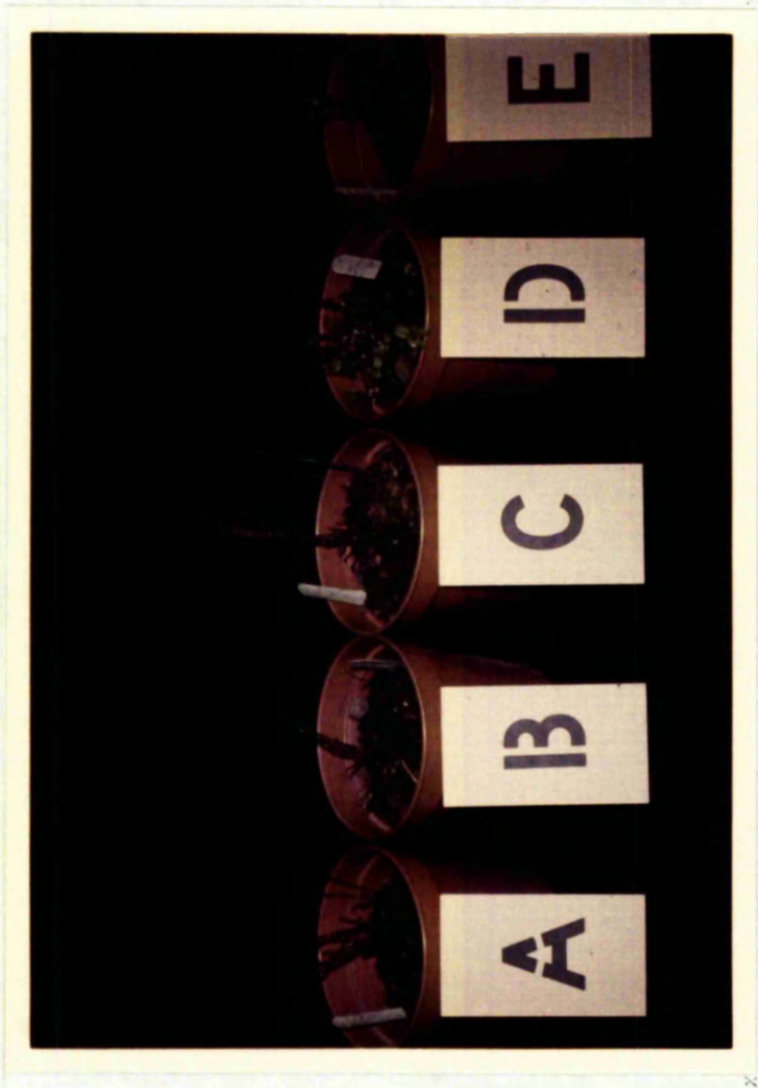


Plate 11. Bud-break and subsequent growth in Vaccinium myrtillus
after 21 days. A-C at 5°C, D-E at 20°C.

See text for details.

Photograph taken after 21 days shows new growth in D and E, but none in A - C (Plate 11).

Vaccinium vitis-idaea

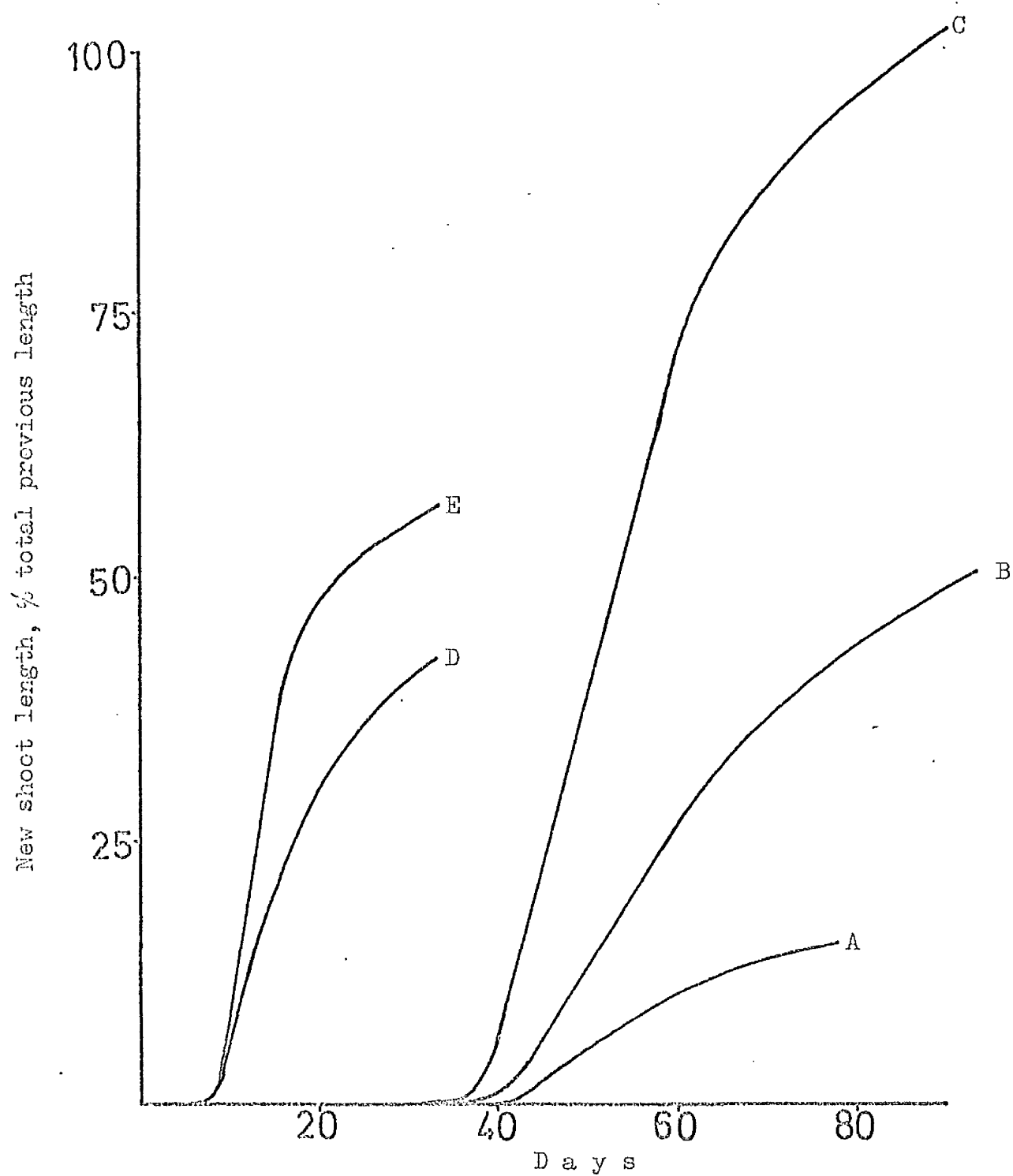
It was not possible to obtain bud-break information in this species since quite often only terminal buds broke and the number of terminal buds was often very small (less than 4). It was possible, however, to obtain the time to first appearance of bud-break at each treatment, and an analysis of variance has been carried out. One replicate was missing from treatment E, making an estimate necessary, thus losing one degree of freedom. Results are given in Table 23. $F = 64.460^{***}$ ($p < 0.001$) is very highly significant, and the least significant difference between means is 5.85. As with V. myrtillus, significant differences are seen between low (A, B and C) and high (D and E) temperature regimes, and also between means of different light regimes at the low temperature level.

Table 23. Analysis of variance between time to appearance of bud-break in treatments A to E - Vaccinium vitis-idaea

Source of Variation	Degrees of Freedom		Sum of Squares		Mean Square	Variance Ratio (F)
Replicates	3		12.55		4.186	0.305
Treatments	4		3544.00		886.00	64.460 ^{***}
Residual	11		151.20		13.745	
Total	18 (19-1)		3707.75			
Treatment	A	B	C	D	E	Least significant difference
Mean	42.0	35.0	28.0	9.5	9.2	5.85

New shoot length is presented in Figure 37. Each point is the

Figure 37. New shoot length, V. vitis-idaea



- A : 8°C, 8 hr. light
B : 5°C, 12 hr. light
C : 5°C, 18 hr. light
D : 20°C, 8 hr. light
E : 20°C, 16 hr. light



Plate 12. Growth in Vaccinium vitis-idaea after 21 days (light green shoots in pots D and E). A-C at 5°C, D-E at 20°C.

See text for details.

mean of two values. The difference between the two temperature groups is evident and, as in Vaccinium uliginosum, differences in shoot length in A, B and C are small to begin with, but are very large at the end of the experiment. Also shoot length in E is greater than D at the end of the experiment but is the same at the beginning.

Photograph taken after 21 days shows new green leaves (light green) in D and E but no new leaves in A - C (Plate 12).

Discussion

It would appear from the results that the two factors, temperature and daylength, influence bud-break in different ways. In V. uliginosum, temperature alone appears to be the factor influencing bud-break, but in V. myrtillus and V. vitis-idaea, both temperature and daylength appear to be operating. Although significant differences in the time to bud-break are observed in the latter species between different temperature groups, increasing daylength appears to have a significant effect in promoting bud-break at the lower temperature. This effect is not observed at the high temperature treatments.

The significant difference in shoot length of all three species at the end of the experiment between treatments A, B and C is thought to be a nutritional effect. As the daylength increases, the length of time at which photosynthesis operates also increases. More photosynthetic products are available with the longer photoperiods and thus growth is enhanced (e.g. treatment C), whereas growth is retarded by lack of products at short photoperiods (e.g. treatment A). The greatest difference in treatments A, B and C due to this effect is seen with the ever-

green V. vitis-idaea (Figure 37) which also, at the higher temperature, has a greater shoot length in E than in D at the end of the experiment.

Anthocyanins were produced in the longer daylength under low temperatures but no anthocyanin was observed in the leaves at short days (treatment A).

There is some evidence to suggest that in treatments A, B and C plants of V. uliginosum have fully matured before the end of the experiment (Figure 33), as the graphs have levelled off near the end of the period. In V. myrtillus and V. vitis-idaea, however, plants at the end of the experiment in treatments A - C are still continuing to grow, indicating that they have not reached maturity (Figures 36 and 37). This, to some extent, substantiates the suggestion made in the frost resistance discussion that short seasons due to low temperatures at high altitude cause the failure of V. myrtillus and V. vitis-idaea to mature properly, whereas V. uliginosum is able to mature fully.

Frost hardening experiment

Table 24 presents the frost-hardiness of V. myrtillus and V. vitis-idaea at each of the four treatments after 62 days.

Table 24. Frost hardiness (t_{50} , °C) of Vaccinium myrtillus and V. vitis-idaea after treatment

<u>Treatment</u>	<u>V. myrtillus</u>	<u>V. vitis-idaea</u>
I (cold frame)	-9.8	-6.5
II (18°C, 6 + 2 hrs light)	-9.4	-9.8
III (5°C, 8 + 2 x 4 hrs light)	-11.9	-11.9
IV (5°C, 6 + 2 hrs light)	-13.6	-11.3
Least significant difference	1.3°C	0.9°C

It appears that the greatest frost hardiness in both species has developed in treatments III and IV at 5°C, little difference between the latter two being shown by V. vitis-idaea but V. myrtillus being more hardy at short days and low temperatures in IV. After these treatments, V. vitis-idaea from treatment IV was placed for three weeks in the dark at 5°C. Hardiness of -15.1°C was induced by this means.

An analysis of variance (see Appendix 3) between the treatments was carried out for both species and results are presented in Tables 25 and 26. The loss of two degrees of freedom represents the estimate of two missing values in Table 25.

Table 25. Analysis of variance between t_{50} values - V. myrtillus

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	Variance Ratio (F)
Replicates	1	5.08	5.08	0.69
Freezing (F)	5	21433.40	4286.68	586.57***
Treatment (T)	3	1289.38	429.79	58.81***
F x T	15	1543.99	102.93	14.08***
Residual	21	153.47	7.31	
Total	45 (47-2)	24425.32		

Least significant difference = 5.65 units of freezing index (I)

≡ 1.3°C at the 50% level of I

Table 26. Analysis of variance between t_{50} values - V. vitis-idaea

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	Variance Ratio (F)
Replicates	1	43.15	43.15	2.73
Freezing (F)	5	49888.05	9977.61	631.29***
Treatment (T)	3	1427.65	475.88	30.11***
F x T	15	5022.49	334.83	21.18***
Residual	23	363.51	15.81	
Total	47	56744.85		

Least significant difference = 8.19 units of freezing index (I)

= 0.9°C at the 50% level of I.

From the analyses, it is seen that the freezing x treatment interaction is very highly significant in both V. myrtillus and V. vitis-idaea. This indicates that the reaction of the tissues to freezing level significantly varies with treatment, showing that variation between t_{50} values determined at each treatment also is very highly significant (see Appendix 3). The least significant difference between any pair of freezing x treatment interaction means has been calculated for each species (see Appendix 3), expressed in °C and is presented in Table 24.

From this table, it is seen that in both species, frost hardiness in treatments I and II, at high temperatures, differs significantly from hardiness in treatments III and IV at low temperatures. In V. myrtillus, significant differences in hardiness between light regimes is observed at the low temperature only, whereas in V. vitis-idaea significant differences are seen only at the high temperatures.

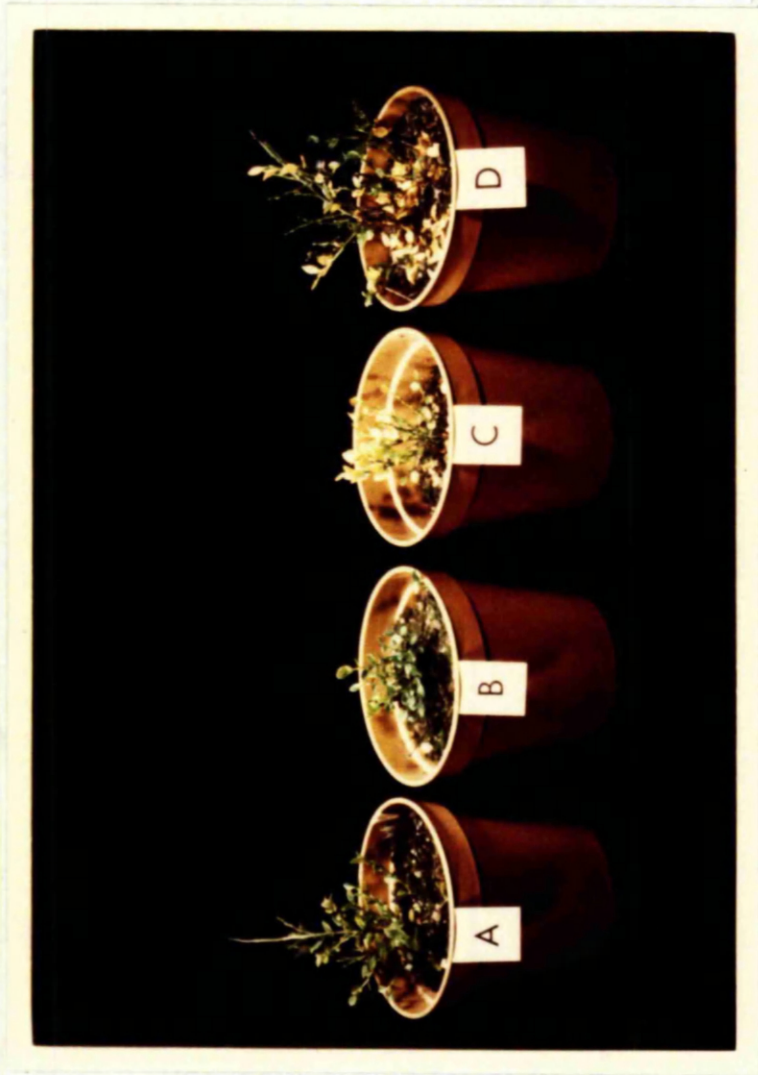


Plate 13. Vaccinium myrtillus after 62 days hardening treatment.

A: cold frame	C: 5°C, 8+2x4 hrs light
B: 18°C, 6+2 hrs light	D: 5°C, 6+2 hrs light



Plate 14. Vaccinium uliginosum after 62 days hardening treatment.

A: cold frame.	C: 5°C, 8+2x4 hrs light
B: 18°C, 6+2 hrs light	D: 5°C, 6+2 hrs light

This indicates that for V. myrtillus, long daylengths at low temperatures have not prevented hardening taking place, but this hardening is not as great as in short days and low temperatures. Short daylengths at high temperatures have no influence on hardening in V. myrtillus compared with the control in the cold frame.

However, in V. vitis-idaea, a significant increase in hardness is observed at high temperatures due to short days, but no difference is observed at low temperatures between long and short daylengths.

Table 27 gives the leaf status in V. myrtillus and V. uliginosum at the end of the experiment. Leaves are expressed as percentage of total counted.

Table 27. State of leaves on shoots of V. myrtillus and V. uliginosum
after treatment

Treatment	<u>V. myrtillus</u>			<u>V. uliginosum</u>		
	green	yellow	fallen	green	yellow	fallen
I (cold frame)	100	0	0	100	0	0
II (18°C, 6 + 2 hrs light)	100	0	0	100	0	0
III (5°C, 8 + 2 x 4 hrs light)	8.9	54.4	36.7	32.3	67.7	0
IV (5°C, 6 + 2 hrs light)	25.7	13.6	60.7	0.8	96.0	3.2

Plates 13 and 14 show the condition of V. myrtillus and V. uliginosum after treatment (62 days). Pots A, B, C and D are equivalent to treatments I, II, III and IV respectively.

It is seen from both Table 27 and Plates 13 and 14 that yellow leaves on the plants and fallen leaves only developed in both species at those treatments with low temperatures, the greatest number fallen occurring

in treatment IV under short days. It is also noted that the leaves fall more readily from V. myrtillus than they do from V. uliginosum.

Discussion

From the results, the varying influences of temperature and day-length are seen. In general for both V. myrtillus and V. vitis-idaea low temperatures induce hardening. Long days prevent this to a certain extent (in V. myrtillus) whereas short days at high temperatures also induce hardening to a certain extent, (in V. vitis-idaea).

Since yellowing of leaves and abscission indicate the onset of dormancy, it is interesting to see that the greatest frost hardiness in V. myrtillus coincides with the greatest abscission of leaves, an indication perhaps of the relationship between the onset of dormancy and the development of frost hardiness.

(e) General Discussion

The first experiment has shown that bud-break in V. uliginosum is entirely influenced by temperature, whereas in V. myrtillus and V. vitis-idaea, photoperiod is operative at low temperatures. Photoperiod at low temperatures also has a significant effect on the subsequent growth of all three species.

The frost hardening experiment has shown that short days at 18°C induced hardening in V. vitis-idaea but not to the same extent as short days at 5°C. Long days at 5°C prevented full hardening from developing in V. myrtillus compared with short days at 5°C. The symptoms of the onset of dormancy in V. myrtillus and V. uliginosum occurred at the low

temperature treatments only.

The greater length of growing season at lower latitudes, with longer daylengths before the Vernal Equinox and despite the similarity of mean temperatures with more northern latitudes, (see frost resistance section) is supported by the growth cabinet studies in which -

- (a) longer daylengths promote earlier bud-break in V. myrtillus at the same low temperature, and
- (b) longer daylengths prevent hardening from fully developing in V. myrtillus.

The suggestion that short seasons cause immaturity in V. myrtillus and V. vitis-idaea on Ben Lui (see frost resistance section) is also supported by growth cabinet studies in which after 85 days, these two species are continuing to grow at low temperatures, whereas V. uliginosum has stopped growing.

PART IV - GENERAL DISCUSSION AND CONCLUSIONS

Schimper (1898, English translation 1903) pointed out that the ecology of plant distribution would in the future lean heavily on experimental physiology, and this approach has been taken up by many workers, some of whom are referred to in previous sections.

The present study of the frost and drought resistance of three Vaccinium species has revealed relationships between several aspects of their distribution and their response to environmental factors. These conclusions have been fully discussed in previous sections and are now listed below.

- (1) The most important environmental factor influencing the frost hardiness of the Vaccinium species has been found to be daylength.
- (2) V. myrtillus is thought to have a longer growing season at more southern latitudes because of daylength (see frost resistance section and Figure 18). This is supported by
- (3) growth cabinet studies, in which although temperature had a significant effect on bud-break in V. myrtillus and V. vitis-idaea, the longer photoperiods at 5°C induced earlier bud-break, and
- (4) growth cabinet studies, in which long photoperiods at 5°C retarded the development of frost hardiness in V. myrtillus.
- (5) Growth cabinet studies have shown that the rate of growth after bud-break is faster at 20°C than at 5°C. At 5°C, the final growth of all three Vaccinium species was greatest at the

longest photoperiod. Photoperiod is thought to have a nutritional effect.

- (6) Daylength, although not statistically significant, has also been found to be the predominant environmental factor influencing the variation in drought resistance of V. vitis-idaea, but it is thought that this relationship between photoperiod and drought resistance is complex. Drought resistance is also affected by age.
- (7) Temperature has been found predominant in influencing the seasonal frost hardiness of V. uliginosum, which hardens to a maximum of -33°C compared with a maximum of -15°C for the other two Vaccinium species on Ben Lui.
- (8) Growth cabinet studies have also shown that bud-break in V. uliginosum is influenced by temperature alone.
- (9) Temperature has also been found to predominantly influence the seasonal variation in frost hardiness of V. myrtillus from Garelochhead. This is thought due to some effect of shading on the growth and development of the plant. This explanation is also thought responsible for
- (10) the poor stomatal control shown by this species (drought resistance section), compared with the open site at Milngavie Moor.
- (11) Rainfall has been found to be a factor significantly influencing the frost hardiness of V. myrtillus, Milngavie Moor, and V. myrtillus and V. vitis-idaea, Loch Katrine. It also extensively contributes to drought resistance in V. vitis-idaea,

Malham Tarn and Loch Katrine. Low rainfall in all cases induced an increase in hardiness.

- (12) It was found, that the maximum frost hardiness developed in V. myrtillus and V. vitis-idaea at 2,250 ft. (765 m) on Ben Lui was less by about 5°C than the hardiness developed in the same species at low altitudes. It is thought that there is an inefficient response to hardening stimuli, because of immaturity due to the short growing season at the higher altitudes (see frost resistance section and Plate 10). This idea is supported by
- (13) growth cabinet studies, in which growth of V. myrtillus and V. vitis-idaea was continuing after 85 days at 5°C, whereas V. uliginosum had stopped growing.
- (14) Because of (12), snow cover is thought essential for survival of V. myrtillus and V. vitis-idaea at high altitudes. Frost hardiness of V. vitis-idaea, Ben Lui, turf form, under snow, has been found to be less than minimum air temperature in spring 1969 (Figure 11).
- (15) Short days at 18°C have induced hardening in V. vitis-idaea (growth cabinet studies).
- (16) The greatest hardiness induced in the growth cabinet studies was -15.1°C in V. vitis-idaea, kept at 5°C and short days for nine weeks with a further three weeks in the dark at 5°C.
- (17) V. vitis-idaea, Ben Lui, turf form, apart from exhibiting xeromorphic characteristics (Table 1), also exhibits a higher transpiration rate, lower relative water contents at stomatal closure

and a greater drought hardiness than V. vitis-idaea, Ben Lui, ledge form. V. vitis-idaea has thus adapted to the more xeric turf habitat, both by reduction of the phenotype and also by physiological adaptations.

- (18) V. myrtillus, Ben Lui, turf form, also exhibiting xeromorphic characteristics, only differs physiologically from the ledge form when plants are more mature.
- (19) V. uliginosum exhibits a similar physiological behaviour to the large-leaved V. myrtillus.
- (20) It has been possible to distinguish between the more xeromorphic species, V. vitis-idaea, and the mesomorphic or hygromorphic species of V. myrtillus and V. uliginosum.
- (21) V. vitis-idaea forms forgo a degree of stomatal closure in the spring which effectively lengthens the period of assimilation by allowing gaseous exchange to take place over a wide range of water deficits.
- (22) In general, the periods of maximum frost and drought hardiness of V. vitis-idaea correspond well, the greater hardiness of both being in winter (cf. Pisek and Larcher, 1954). However, the situation appears to be more complex when specific individual values are considered, and therefore no significant correlation is observed. It thus appears that frost and drought hardiness of V. vitis-idaea show a differential response to changes in environmental factors.

Metcalf (1950) and Burges (1951) both have suggested that snow-

cover is essential for survival of V. myrtillus in the Cairngorm massif, and it competes less successfully with Calluna at the lower limit of snow protection [about 3,125 ft. (950m)] (Burgess, 1951). In Scandinavia, snow cover is thought to determine the altitudinal limit of both V. vitis-idaea and V. myrtillus, (see Ritchie, 1955; 1956), frost damage to uncovered plants being the limiting factor. Although it has been suggested that frost-drought damage is responsible for limitations (e.g. Michaelis, 1934; Tranquillini, 1967; Havas, 1969), the present study has substantiated the suggestion of Metcalfe (1950) and Scandinavian workers (see Ritchie, 1956) that damage could be due to frost.

Although frost hardiness levels in V. myrtillus and V. vitis-idaea at high altitude [2,250 ft. (765 m)] on Ben Lui remained greater than the minimum air temperatures, levels were less than minimum air temperatures on four occasions [see (14) above]. It is probable, also, that minimum temperatures at ground level are lower than minimum air temperatures, due to a micro-climate effect (see Figure 1, 1-5 hours). No damage was observed in this turf form of V. vitis-idaea because of the protection of snow cover. It is thought that although maximum frost hardiness in high altitude forms is not as great as lowland forms, this in itself does not cause limitations to the species at the altitude studied, but since daylength has such a marked effect on variation in frost hardiness, de-hardening caused by longer daylengths will begin from a lower level of frost hardiness. Thus in late spring, before the new growth sets in, when frosts are still common, the plants will be susceptible to damage, especially if not covered by snow. Altitudinal limits are thus thought

to be set by frost damage in the spring, occurring in V. myrtillus and V. vitis-idaea not covered by snow. V. vitis-idaea appears to lose hardiness earlier than V. myrtillus (Figures 10 and 11) and may be more susceptible for a longer period. Maximum levels of hardiness reached at higher altitudes are unlikely to differ greatly from those attained in the study area [2,250 ft. (765 m)], and may also be the cause of altitudinal limitations in areas of little or no snow cover.

The greater length of growing season, and the longer winter daylength (causing an earlier dehardening) at more southern latitudes [see (2)-(4) above] mean that V. myrtillus will be potentially more susceptible to early and late frosts, and this may cause altitudinal limits to its distribution, especially by the possible frost damage to new growth in the spring. No latitudinal limits to its distribution, however, are thought due to this factor. No evidence has been found to explain the southern limit of V. vitis-idaea. The possibility of an altitudinal limit by damage to young growth as mentioned above is supported by Zobel (1969) who found that Pinus pungens appeared to be limited altitudinally by low temperature injury to new growth.

Although V. uliginosum is at its most southern limit in Britain, it does not appear to have lost its capacity to harden against frost damage. As mentioned in the frost resistance section, V. myrtillus and V. vitis-idaea generally appear to have a maximum frost hardiness which is much less than hardy arctic-alpine plants. It would seem, therefore, that capacity to frost harden is genetically determined, though woodiness may have a small effect - V. uliginosum being woodier than the other two species.

It is not known what causes the latitudinal or altitudinal limit of V. uliginosum in Britain.

Ritchie (1955) suggests that V. vitis-idaea shows some preference for well-drained slopes, and from the present study this may be due to the fact that this species is xeromorphic in character and thus better adapted to drier habitats, especially the turf form which exhibits greater xeromorphic characteristics [see (17) above], as well as greater physiological adaptations to xeric habitats. It is thought that the restriction of V. uliginosum to wet montane ledges in the area studied, is due to the more hygrophytic characteristics of this species and a probable inability to compete successfully for water when this is in short supply e.g. on the turf habitat.

The usefulness of relative water contents in measuring stomatal closing points and drought hardiness has been demonstrated, and it has been possible in this study to distinguish between different habitats and different species of Vaccinium using these techniques.

Growth cabinet studies have demonstrated how well growth and physiology can be controlled and measured, and it has been possible to relate the physiological findings from these artificial studies to the ecological situation in the field.

Apart from revealing aspects of the distribution of the three Vaccinium species and their relationship to the environment, this study has shown how artificially controlled environment studies and physiological measurements of field material can contribute to an understanding of the ecology of certain plant species.

APPENDIX 1. The effect of moisture content on the frost
hardiness of *Vaccinium vitis-idaea*
Loch Katrine, collected 17-12-68

In Levitt's review (1956), it is shown that many workers have found an inverse relationship between moisture content and frost hardiness while many others have found no such relationship. If moisture content does affect frost hardiness, inaccurate measurements may arise due to water deficits in the plant. An experiment was set up to examine the effect of water content on frost hardiness of *V. vitis-idaea*, Loch Katrine.

Seventy two shoots of *V. vitis-idaea* were allowed to saturate by standing overnight in water in glass jars. Four samples of nine plants were then selected at random, each plant weighed, and the samples placed in an incubator at $28 \pm 1^{\circ}\text{C}$ under a 100 watt bulb for 1/2 hour, 1 hour and 2 hours. One sample was not subjected to this drought treatment.

After the drying treatment, each plant from the three treated groups was reweighed, placed in glass containers and transferred to a cooling bath at -7.2°C (see section on frost resistance methods). The drying procedure was arranged so that only one sample of 9 plants was in the bath at the one time. The rate of cooling is very rapid (cf. frost resistance discussion) because direct transfer to the freezing temperature was necessary. Each sample was allowed to equilibrate and left at the freezing temperature for 3/4 hour, after which it was removed and individual plants thawed, cut up and placed in 25 ml. distilled water.

Table A. Damage at two temperatures and four moisture contentsV. vitis-idaea, Loch Katrine, 17-12-68

Temperature	-7.2°C			-11.1°C	
Moisture content	Replicates	Total of 9	Total of 18	Total of 9	Replicates
I (100%)	20.000 17.088 26.950 28.000 38.157 24.186 17.647 34.690 32.941	239.659	946.683	707.024	70.238 78.921 85.211 82.142 86.885 78.064 64.202 79.057 82.304
II (87%)	20.000 17.948 10.138 13.658 27.956 18.644 21.379 20.338 24.719	174.780	567.297	392.517	43.537 70.873 37.719 47.445 36.054 30.000 36.000 40.404 50.485
III (83%)	35.928 28.627 21.461 24.175 32.352 22.513 22.580 16.578 19.211	223.425	698.556	475.131	45.641 52.127 59.863 71.631 52.459 47.161 58.102 38.912 49.235
IV (76.5%)	52.083 57.073 26.008 35.714 33.598 25.454 32.558 28.571 29.687	309.746	771.631	461.885	48.226 39.597 48.447 48.181 56.896 66.889 49.333 49.866 54.450
Total		947.610	2,984.167	2,036.557	

Conductance measurements and autoclaving were made as described in section on frost resistance methods. This procedure was repeated for a second set of 4 samples at a freezing temperature of -11.1°C . Dry weights of individual plants were finally determined, and relative water contents were calculated. The mean relative water content of each sample was then calculated. Indices of damage were calculated for each plant (frost resistance section) and the results are presented in Table A. The means are presented in Table B.

Table B. Means of indices of injury at two temperatures and four moisture contents

Moisture content		Temperature	
		-7.2°C	-11.1°C
I	100%	26.629	78.558
II	87%	19.420	43.613
III	83%	24.825	52.792
IV	76.5%	34.416	51.321

Least significant difference = 8.39 units of injury

Analysis of variance was carried out on results in Table A, and results are presented in Table C. Replicates contribute no significant variation to the analysis, and the interactions of replicates with other factors are included in the residual (error) sum of squares.

Table D. Analysis of variance after partitioning

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	Variance Ratio (F)
Replicates	8	492.197	61.525	0.777
Temperature	1	16,466.855	16,466.855	207.962***
Water level				
(a) I versus rest	1	2,979.327	2,979.327	37.626***
(b) within rest	2	1,191.134	595.567	7.521***
sub-total	3	4,170.461		
W x T				
(a) I versus rest	1	2,823.039	2,823.039	35.652***
(b) within rest	2	284.634	142.317	1.797 ns
sub-total	3	3,107.673		
Residual	56	4,434.211	79.182	
Total	71	28,671.397		

Table C. Analysis of variance of results in Table A.

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	Variance Ratio (F)
Replicates	8	492.197	61.525	0.777
Water levels (W)	3	4,170.461	1,390.154	17.556***
Temperature (T)	1	16,466.855	16,466.855	207.962***
W x T	3	3,107.673	1,035.891	13.082***
Residual	56	4,434.211	79.182	
Total	71	28,671.397		

*** = ($p < 0.001$)

Least significant difference between
any pair of W x T means = 8.39 units of injury

Interaction is seen between temperature and water level and this was thought to be due to the variable effects of temperature at the different water levels. Inspection of Table A reveals no pattern to this interaction, but it is observed that the figure for 100% RWC at -11.1°C (707.024) is rather large compared with the others. The significant interaction might be due to an interaction between I and the other water levels. Sums of squares of water levels and W x T interaction were partitioned, and results presented in Table D.

From Table D, a significant interaction is observed between I and the rest, with non-significance being observed within water levels II - IV. This indicates the source of significance of the W x T interaction in Table C.

Discussion

A highly significant water level x temperature interaction is observed between those treatments with 100% moisture content and those with lower moisture contents.

Inspection of Table B indicates that lower water contents modify the increase in damage due to freezing observed in the fully saturated treatments.

When damage due to freezing is slight in fully saturated material, lower moisture contents appear to have little effect in reducing this damage. When damage due to freezing is extensive in saturated material, lower moisture contents have a significant effect in reducing this damage.

It is therefore concluded that lower moisture contents significantly reduce frost damage to the tissue of Vaccinium vitis-idaea only at temperatures which are likely to cause extensive damage.

APPENDIX 2. Plant species quoted in text

The specific names of several of the species quoted in the text have been altered since the date of the research quoted. A list of the names of all species with authorities is given below and the present name included if different.

Floras by Bailey (1949), Clapham et al. (1962), Dallimore and Jackson (1966), Watson (1968) and Flora Europaea (1964, 1968) have been referred to. Names are in alphabetical order.

Higher plants

Abelia R. Br.

Acer negundo L.

Betula L. spp.

Calluna vulgaris (L.) Hull

Chrysopogon Trin.

Citrus sinensis Osbeck

Convallaria majalis L.

Corylus avellana L.

Empetrum nigrum L.

Erica cinerea L.

Erica tetralix L.

Festuca ovina L.

Fraxinus excelsior L.

Loiseleuria procumbens (L.) Desv.

Maianthemum bifolium (L.) Schmidt

Higher plants (contd)

Melampyrum pratense L.

Picea excelsa Link. = Picea abies (L.) Karst

Pinus cembra L.

Pinus montana Miller = Pinus mugo Turra

Pinus pungens Lamb. ex Michx. f.

Platanus occidentalis L.

Populus deltoides Marshall

Quercus L. spp.

Quercus ilex L.

Rhododendron ferrugineum L.

Salix herbacea L.

Saxifraga oppositifolia L.

Saxifraga stellaris L.

Sedum rosea (L.) Scop.

Silene acaulis (L.) Jacq.

Stipa L.

Ulex europaeus L.

Ulex gallii Planch.

Vaccinium myrtillus L.

Vaccinium uliginosum L.

Vaccinium vitis-idaea L.

Viburnum plicatum var. tomentosum Miq.

Pteridophytes

Pteridium aquilinum (L.) Kuhn.

Bryophytes

Racomitrium lanuginosum (Hedw.) Brid.

APPENDIX 3. Statistical methods

Analysis of variance

In the frost resistance section and growth cabinet studies, hardening experiment, it was necessary to distinguish variation in t_{50} values from random variation in the sampled populations. No direct way of comparing t_{50} values was discovered but it was found possible to compare indices of injury at different freezing levels at various times throughout the year. Five freezing levels were common to all determinations throughout the whole year - $+5^{\circ}\text{C}$, -4°C , -8°C , -12°C and -16°C and the indices of injury at each of these levels were compared throughout the year by Analysis of Variance, using methods of calculation given by Snedecor and Cochran (1967).

The alteration of t_{50} with time reflects the reaction of the tissues to freezing level at different times hence a significant interaction between freezing level and time indicates a significant alteration in t_{50} value.

A typical example is given below, though the figures have been simplified for clarity. Figures in brackets are estimates of missing values. A degree of freedom is lost for each estimate.

Since replicates have not been randomized, the sum of squares due to replicates has not been extracted in this analysis. In analysis of growth cabinet studies, sum of squares due to replicates is included since randomization was carried out.

Calculation (see Table A)

$$\text{Correction} = T^2/N = \frac{(5767)^2}{100} = 332582.89$$

Table A. Indices of injury at freezing levels and time. *V. myrtillus*. Garclochhead

Date	2.11.68	2.12.68	13.1.69	8.2.69	10.3.69	6.5.69	1.7.69	11.8.69	7.10.69	15.12.69	Total of 20
Freezing level	Replicates	Replicates	Replicates	Replicates	Replicates	Replicates	Replicates	Replicates	Replicates	Replicates	Total of 2
I 5°C	100 93	91 (94)	90 (97)	99 (100)	100 94	94 89	100 97	99 99	100 98	98 100	198 1,932
II -4°C	76 62	100 64	90 100	99 100	80 79	99 62	38 26	100 98	(87) (87)	93 100	193 1,639
III -8°C	24 24	38 35	93 (75)	77 75	58 53	(35) (36)	25 21	22 20	29 25	63 60	123 888
IV -12°C	44 34	30 24	55 43	58 47	56 43	16 15	30 28	19 22	27 22	47 53	100 713
V -16°C	24 22	33 30	45 44	45 39	40 33	(12) (11)	30 24	22 22	22 22	36 39	75 595
Total of 10	503	539	732	739	636	469	419	523	518	689	5,767

- (1) Total sum of squares (X = individual index of injury)

$$\sum X^2 - T^2/N = 95854.11$$

- (2) Freezing level

$$\text{Sum of squares} = \frac{(1932)^2 + \dots + (595)^2}{20} - T^2/N = 70911.26$$

- (3) Time

$$\text{Sum of squares} = \frac{(503)^2 + \dots + (689)^2}{10} - T^2/N = 11623.81$$

- (4) Freezing level x times

$$\text{Sum of squares} = \frac{(193)^2 + \dots + (75)^2}{2} - T^2/N = 70911.26 - 11623.81 = 11046.54$$

An analysis of variance table is now set up (Table B) in which all sources of variation given above are compared with the residual variation, this latter being a measure of variation of individual plants.

The variance ratio, $F = \frac{\text{Mean square of item}}{\text{Mean square of residual}}$ is calculated and if F is greater than that value given in tables for the 5% level ($p < 0.05$) then that item contributes significantly to variations in the experiment.

* ($p < 0.05$); ** ($p < 0.01$); *** ($p < 0.001$)

Table B. Analysis of variance table, *V. myrtillus*, Garelochhead

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	Variance Ratio (F)
Freezing level (F)	4	70,911.26	17,727.82	312.06***
Times (T)	9	11,623.81	1,291.53	22.73***
F x T	36	11,046.54	306.85	5.40***
Residual	40	2,272.50	56.81	
Total	89 (99-10)	95,854.11		

Conclusion: The very highly significant interaction between freezing level and times indicates that t_{50} values vary significantly with time.

The residual mean square (56.81) is the sample variance, s^2 of the population.

The standard error of the difference between two means, $\sqrt{\frac{2s^2}{n}}$, is calculated for the freezing level x times means, i.e. the mean of the total of each two replicates.

In the example $n = 2$ and the standard error = $7.537, (\sqrt{56.81})$.

The 5% value of t with 40 degrees of freedom is 2.02. Hence the difference between a specific pair of freezing level x time means is significant at the 5% level if it exceeds $(2.02) (7.537) = 15.22$ units of injury. This value is the least significant difference (LSD) between any pair of interaction means.

In the example, a difference of 15.22 units of injury around the 50% index of injury level is equivalent to a difference of 0.8°C in summer and 2.5°C in winter between t_{50} values. These are estimated from the freezing curves, and the difference between summer and winter occurs because the slope of the graph with which t_{50} is determined is steeper in summer than in winter.

Multiple regression analysis

Additional information about a dependent variable Y is obtained by means of a multiple regression on several X 's.

In the present study, multiple regression is used for -

- (1) constructing an equation in X 's that gives the best prediction of the values of Y ,

Table C. Multiple regression, Vaccinium myrtillus, Ben Iuiledge form

Date	X ₁	X ₂	Y
16. 5.68	4.1	16.3	- 6.2
30. 5.68	9.4	17.1	- 5.1
12. 6.68	13.2	17.5	- 4.3
5. 9.68	8.9	14.1	- 3.9
3.10.68	7.8	12.0	- 6.0
25.11.68	3.8	8.0	-12.1
7. 1.69	1.3	7.1	-11.1
17. 2.69	-3.2	9.5	-12.7
15. 4.69	3.4	13.8	- 7.8
28. 4.69	3.6	14.8	- 8.4
10. 6.69	7.8	17.3	- 6.2
29. 7.69	11.8	16.6	- 3.8
Total	71.9	164.1	-87.6
Mean	5.99	13.67	- 7.30

X₁ = mean temperature (°C)X₂ = mean daylength (hours)Y = t₅₀ value (°C)

$$\sum X_1^2 = 670.430$$

$$C = 430.800$$

$$\sum x_1^2 = 239.630$$

$$\sum X_1 X_2 = 1,117.910$$

$$C = 983.230$$

$$\sum x_1 x_2 = 134.680$$

$$\sum X_1 Y = - 381.360$$

$$C = - 524.870$$

$$\sum x_1 y = 143.510$$

$$\sum X_2^2 = 2,396.250$$

$$C = 2,244.067$$

$$\sum x_2^2 = 152.183$$

$$\sum X_2 Y = -1,089.070$$

$$C = -1,197.930$$

$$\sum x_2 y = 108.860$$

$$\sum Y^2 = 749.340$$

$$C = 639.480$$

$$\sum y^2 = 109.860$$

- (2) discovering which variables are related to Y and rating these in order of importance.

Normally calculations involving three or more X-variables are done on standard electronic computer programs, two of which have been used in the present study. However, an example with two X variables is given below, using the method of calculation of Snedecor and Cochran (1967).

Given a sample of n values of (Y, X₁, X₂) the sample regression or prediction equation is

$$\hat{Y} = a + b_1 X_1 + b_2 X_2$$

$$\text{where } a = \bar{Y} - b_1 \bar{X}_1 - b_2 \bar{X}_2$$

$$b_1 = \frac{(\sum x_2^2)(\sum x_1 y) - (\sum x_1 x_2)(\sum x_2 y)}{D}$$

$$b_2 = \frac{(\sum x_1^2)(\sum x_2 y) - (\sum x_1 x_2)(\sum x_1 y)}{D}$$

$$D = (\sum x_1^2)(\sum x_2^2) - (\sum x_1 x_2)^2$$

Using the above equations and Table C, a = -14.6884, b = 0.3916, b₂ = 0.3687 and $\hat{Y} = -14.6884 + 0.3916X_1 + 0.3687X_2$.

The contribution of regression to variation in Y = $\sum \hat{y}^2 / \sum y^2$

where $\sum \hat{y}^2 = b_1 \sum x_1 y + b_2 \sum x_2 y = 96.335$

$$\text{i.e. } \% \text{ variation due to regression} = \frac{96.335}{109.860} \times 100 = 87.69\%$$

The significance of the regression is calculated from the variance ratio, F, where

$$F = \frac{\text{Regression mean square } (\sum \hat{y}^2 / k-1)}{\text{Deviations or Residual mean square } (\sum y^2 - \sum \hat{y}^2 / n-k)}$$

with $(k-1)$ and $(n-k)$ degrees of freedom (k = total number of variables).

In the example $F = \frac{48.168}{1.5028} = 32.052^{***}$ Variance due to regression is very highly significant ($p < 0.001$).

Regression coefficients cannot be simply compared since a correction for scale is necessary. This correction is $\sqrt{\sum x^2 / \sum y^2}$ and the standard partial regression coefficient (β) so formed = $b \sqrt{\frac{\sum x^2}{\sum y^2}}$.

In the example, the standard partial regression coefficient of X_1

$$\beta_1 = 0.3916 \sqrt{\frac{239.630}{109.860}} = 0.5780$$

and of X_2

$$\beta_2 = 0.3687 \sqrt{\frac{152.183}{109.860}} = 0.4336$$

Comparisons of these gives the contribution of

$$X_1 \text{ to the regression} = \frac{0.5780}{0.5780 + 0.4336} \times 100 = 57.13\%$$

$$X_2 \text{ to the regression} = (100.00 - 57.13) = 42.87\%.$$

Methods of calculating significance of individual coefficients are given in Snedecor and Cochran (1967). Multiple regressions with more than 2 X variables are also considered.

REFERENCES

- Åkerman, Å. (1927). Studien über den Kältetod und die Kälteresistenz der Pflanzen. Lund. Cited by Levitt (1956).
- Alexandrov, V.Y. (1964). Cytophysiological and cytoecological investigations of heat resistance of plant cells toward the action of high and low temperature. Q. Rev. Biol. 39, 35-77.
- Amirshahi, M.C. & Patterson, F.L. (1956). Development of a standard artificial freezing technique for evaluating coldresistance of oats. Agron. J., 48, 181-184.
- Arvidsson, I. (1951). Austrocknungs - und Dürrerresistenz - verhältnisse einiger Repräsentanten öländischer Pflanzenvereine, nabst Bemerkungen über Wasser-absorption durch oberirdische Organe. Oikos, Suppl. 1, 1-181.
- Asahina, E. (1956). The freezing process of plant cell. Contr. Inst. low Temp. Sci., Hokkaido Univ., 10, 83-126.
- Bailey, L.H. (1949). Manual of Cultivated Plants, 2nd Edition, MacMillan Co., New York.
- Bannister, P. (1964). Stomatal responses of heath plants to water deficits. J. Ecol., 52, 151-158.
- Bannister, P. (1970). The annual course of drought and heat resistance in heath plants from an oceanic environment. Flora, Jena, 159, 105-123.
- Barrs, H.D. (1966). Root pressure and leaf water potential. Science, 152, 1266-1268.

- Barrs, H.D. (1968). Determination of water deficits in plant tissues.
In Water Deficits & Plant Growth, Vol. 1. (Kozlowski, T.T.,
ed.), Academic Press.
- Bayles, B.B., Taylor, J.W. and Bartel, A.T. (1937). Rate of water loss
in wheat varieties and resistance to artificial drought.
J. Am. Soc. Agron., 29, 40-52.
- Biebl, R. (1962). Protoplasmatische Ökologie der Pflanzen I. Wasser
und Temperatur. Protoplasmatologia, 12, 1-344.
- Billings, W.D. (1952). The environmental complex in relation to plant
growth and distribution. Q. Rev. Biol., 27, 251-265.
- Billings, W.D. (1957). Physiological ecology. A. Rev. Pl. Physiol.,
8, 375-392.
- Billings, W.D. and Mooney, H.A. (1968). The ecology of arctic and
alpine plants. Biol. Rev., 43, 481-529.
- Bliss, L.C. (1956). A comparison of plant development in micro-environ-
ments of arctic and alpine tundras. Ecol. Monogr., 26,
303-337.
- Bright, D.N.E. (1928). The effects of exposure upon the structure of
certain heath plants. J. Ecol., 16, 323-366.
- Brown, W.V. (1954). Staining of plant cells by tetrazolium chloride.
Bull. Torrey bot. Club, 81, 127-136.
- Burges, A. (1951). The ecology of the Cairngorms III. The Empetrum-
Vaccinium zone. J. Ecol., 39, 271-284.
- de Candolle, A.L. (1855). Géographie Botanique Raisonnée. Paris.

- Carpenter, W.J.G., McGuire, J.J. and Shutak, V.G. (1963). Comparison of autoclaving and boiling as methods for obtaining release of electrolytes from Ilex crenata 'convexa' Mak. shoots and roots. Proc. Am. Soc. hort. Sci., 83, 782-785.
- Carrier, L.E. (1951). A study of methods of determining the extent of frost injury of roses. Proc. Am. Soc. hort. Sci., 58, 350-356.
- Cartellieri, E. (1935). Jahresgang von osmotischen Wert, Transpiration und Assimilation einiger Ericaceen der alpinen Zwergstrauchheide und von Pinus cembra. Jb. wiss. Bot., 82, 460-506.
- Chambers, R. and Hale, H.P. (1932). The formation of ice in protoplasm. Proc. R. Soc., B 110, 336-352.
- Chandler, W.H. (1954). Cold resistance in horticultural plants : A Review. Proc. Am. Soc. hort. Sci., 64, 552-572.
- Chaney, W.R. and Kozlowski, T.T. (1969). Seasonal and diurnal changes in water balance of fruits, cones and leaves of forest trees. Can. J. Bot., 47, 1407-1417.
- Chaplin, C.G. (1948). Artificial freezing tests of peach fruit buds. Proc. Am. Soc. hort. Sci., 52, 121-129.
- Clapham, A.R., Tutin, T.G. and Warburg, E.F. (1962). Flora of the British Isles, 2nd Edition. Cambridge University Press.
- Clements, H.F. (1938). Mechanism of freezing resistance in the needles of Pinus ponderosa and Pseudotsuga mucronata. Res. Stud. Wash. St. Univ., 6, 3-45.

- Cordukes, W.E., Wilner, J. and Rothwell, V.T. (1966). The evaluation of cold and drought stresses of turf grasses by electrolytic and ninhydrin methods. Can. J. Pl. Sci., 46, 337-342.
- Dallimore, W. and Jackson, A.B. (1966). A Handbook of Coniferae and Ginkgoaceae, 4th edition. Edward Arnold Ltd., London.
- Daubenmire, R.F. (1943). Vegetational zonation in the Rocky Mountains. Bot. Rev., 9, 325-393.
- Dexter, S.T., Tottingham, W.E. and Graber, L.F. (1930). Preliminary results in measuring the hardiness of plants. Pl. Physiol., Lancaster, 5, 215-223.
- Dexter, S.T., Tottingham, W.E. and Graber, L.F. (1932). Investigations of the hardiness of plants by measurements of electrical conductivity. Pl. Physiol., Lancaster, 7, 63-78.
- van den Driessche, R. (1969). Influence of moisture supply, temperature, and light on frost-hardiness changes in Douglas-Fir seedlings. Can. J. Bot., 47, 1765-1772.
- Emmert, F.H. and Howlett, S. (1953). Electrolytic determination of the resistance of fifty-five apple varieties to low temperatures. Proc. Am. Soc. hort. Sci., 62, 311-318.
- Evanari, M. and Richter, R. (1937). Physiological and ecological investigations in the wilderness of Judaea. J. Linn. Soc. (Bot.), 51, 333-381.
- Firbas, F. (1931). Untersuchungen über den Wasserhaushalt der Hochmoorpflanzen. Jb. wiss. Bot., 74, 459-696.

- Flint, H.L., Boyce, B.R. and Beattie, D.J. (1967). Index of Injury -
a useful expression of freezing injury to plant tissues as
determined by the electrolytic method. Can. J. Pl. Sci.,
47, 229-230.
- Flora Europaea (1964, 1968). Six editors. Cambridge University Press.
- Fraser, D.A. and Dirks, H.T. (1959). Internal water relations of yellow
birch at Chalk River. Can. J. Bot., 37, 789-799.
- Geiger, R. (1965). The Climate near the Ground, 4th edition. Harvard
University Press.
- Griggs, R.F. (1946). The timberlines of northern America and their
interpretation. Ecology, 27, 275-289.
- Halevy, A.H. and Monselise, S.P. (1963). Meaning of apparent midnight
decrease in water content of leaves. Bot. Gaz., 124,
343-346.
- Halma, F.F. (1933). Some phases in the water relations of Citrus.
Proc. Am. Soc. hort. Sci., 31, 108-109.
- Hampton, D.A. (1967). The physiology of flowering in Pisum sativum
(the Garden Pea). Ph.D. Thesis, Glasgow University.
- Harvey, R.B. (1918). Hardening process in plants and developments
from frost injury. J. agric. Res., 15, 83-112.
- Hatakeyama, I. and Kato, J. (1965). Studies on the water relations
of Buxus leaves. Planta (Berl.), 65, 259-268.
- Havas, P. (1969). Water economy of blueberry (Vaccinium myrtillus)
during the winter. (Abstract). Rep. 9th Int. Bot.
Congr., p.87. Seattle, Washington.

Henckel, P.A. (1964). Physiology of plants under drought. A. Rev.

Pl. Physiol., 15, 363-386.

Hiesey, W.M. and Milner, H.W. (1965). Physiology of ecological races and species. A. Rev. Pl. Physiol., 16, 203-216.

Höfler, K. (1950). Über Trockenhärtung des Protoplasmas. Ber. dt. bot. Ges., 63, 3-10.

Höfler, K., Misch, H. and Rottenburg, W. (1941). Über die Austrocknungsresistenz landwirtschaftlicher Kulturpflanzen. Forschungsdienst, 12, 50-61.

Huber, B. (1924). Die Beurteilung des Wasserhaushaltes der Pflanzen. Jb. wiss. Bot., 64, 1-120.

Hutchinson, T.C. (1970). Lime chlorosis as a factor in seedling establishment on calcareous soils. II. The development of leaf water deficits in plants showing lime-chlorosis. New Phytol., 69, 143-157.

Huysee, van R.B., Weiser, C.J. and Li, P.H. (1967). Cold acclimation in Cornus stolonifera under natural and controlled photoperiod and temperature. Bot. Gaz., 128, 200-205.

Hygen, G. (1951). Studies in plant transpiration. I. Physiologia Pl., 4, 57-183.

Hygen, G. (1953a). Studies in plant transpiration. II. Physiologia Pl., 6, 106-133.

Hygen, G. (1953b). On the transpiration decline of excised plant samples. Skr. norske Vidensk-Akad. Mat.-naturv. Kl., 1, 1-84.

- Iljin, W.S. (1930). Die Ursachen der Resistenz von Pflanzenzellen gegen Austrocknen. Protoplasma, 10, 379-414.
- Iljin, W.S. (1957). Drought resistance in plants and physiological processes. A. Rev. Pl. Physiol., 8, 257-274.
- Irving, R.M. and Lanphear, F.O. (1967a). Environmental control of cold hardiness in woody plants. Pl. Physiol., Lancaster, 42, 1191-1196.
- Irving, R.M. and Lanphear, F.O. (1967b). The long day leaf as a source of cold hardiness inhibitors. Pl. Physiol., Lancaster, 42, 1384-1388.
- Irving, R.M. and Lanphear, F.O. (1967c). Dehardening and the dormant condition in Acer and Viburnum. Proc. Am. Soc. hort. Sci., 91, 699-705.
- Jarvis, P.G. and Jarvis, M.S. (1963a). The water relations of tree seedlings IV. Some aspects of the tissue water relations and drought resistance. Physiologia Pl., 16, 501-516.
- Jarvis, P.G. and Jarvis, M.S. (1963b). Effects of several osmotic substrates on the growth of Lupinus albus seedlings. Physiologia Pl., 16, 485-500.
- Kappen, L. (1964). Untersuchungen über den Jahreslauf der Frost-, Hitze- und Austrocknungsresistenz von Sporophyten einheimischer Polypodiaceen (Filicinae). Flora, Jena, 155, 123-166.
- Kaszkurewicz, A. and Fogg, P.J. (1967). Growing seasons of cottonwood and sycamore as related to geographic and environmental factors. Ecology, 48, 785-92.
- Klikoff, I.G. (1965). Microenvironmental influence on vegetational pattern near timberline in the Central Sierra Nevada. Ecol. Monogr., 35, 187-211.

- Kneen, E. and Blish, M.J. (1941). Carbohydrate metabolism and winter hardiness of wheat. J. agric. Res., 62, 1-26.
- Kohn, H. and Levitt, J. (1965). Frost hardiness studies on cabbage grown under controlled conditions. Pl. Physiol., Lancaster, 40, 476-480.
- Kozlowski, T.T. (1964). Water Metabolism in Plants. Harper and Row, New York.
- Kozlowski, T.T. (editor) (1968). Water Deficits and Plant Growth, Volumes I and II. Academic Press, New York.
- Kramer, P.J. (1937a). Photoperiodic stimulation of growth by artificial light as a cause of winter killing. Pl. Physiol., Lancaster, 12, 881-883.
- Kramer, P.J. (1937b). The relation between the rate of transpiration and the rate of absorption of water by plants. Ann. J. Bot., 24, 10-15.
- Kramer, P.J. (1949). Plant and Soil Water Relationships. McGraw-Hill Book Company Inc., New York.
- Kramer, P.J. (1963). Water stress and plant growth. Agron. J., 55, 31-35.
- Kramer, P.J. and Brix, H. (1965). Measurement of water deficit in plants. Arid Zone Res., 25, 343-351.
- Larcher, W. (1957). Frost-trocknis an der Waldgrenze und in der alpinen Zwergstrauchheide. Veroff. Mus. Ferdinandeum Innsbr., 37, 49-81.
- Larcher, W. (1963). Zur Frage des Zusammenhanges zwischen Austrocknungsresistenz und Frosthärte bei Immergrünen. Protoplasma, 57, 569-87.

- Larcher, W. (1969). Zunahme des Frostabhärtungsvermögens von Quercus ilex im Laufe der Individualentwicklung Planta (Berl.), 88, 130-135.
- Larcher, W. and Eggarter, H. (1960). Anwendung des Triphenyltetrazoliumchlorids zur Beurteilung von Frostschäden in verschiedenen Achsengeweben bei Pirus - Arten, und Jahresgang der Resistenz. Protoplasma, 51, 595-619.
- Levitt, J. (1956). The Hardiness of Plants. Academic Press, New York.
- Levitt, J. (1958). Frost, drought and heat resistance. Protoplasmatologia, 8, 1-87.
- Levitt, J. (1965). The measurement of drought resistance. Arid Zone Res., 25, 407-412.
- Levitt, J. (1966). Winter hardiness in plants. In Cryobiology (Meryman, H.T., ed.), 495-563. Academic Press, London and New York.
- Li, P.H., Weiser, C.J. and van Huystee, R. (1966). The relation of cold resistance to the status of phosphorus and certain metabolites in red-osier dogwood (Cornus stolonifera Michx.). Pl. Cell Physiol., Tokyo, 7, 475-484.
- Lopushinsky, W. (1969). Stomatal closure in conifer seedlings in response to leaf moisture stress. Bot. Gaz., 130, 258-263.
- Lundegardh, H. (1925). Klima und Boden in ihrer Wirkung auf das Pflanzenleben. 2nd edition. Gustav Fischer, Jena.
- Lundegardh, H. (1931). Environment and Plant Development (translation by E. Ashby). Edward Arnold & Co., London.

- McGuire, J.J. and Flint, H.L. (1962). Effects of temperature and light on frost hardiness of conifers. Proc. Am. Soc. hort. Sci., 80, 630-635.
- Macklon, A.E.S. and Weatherley, P.E. (1965). Controlled environment studies of the nature and origins of water deficits in plants. New Phytol., 64, 414-427.
- Mattas, R.W. and Pauli, A.W. (1965). Trends in nitrate reduction and nitrogen fractions in young corn (Zea mays) plants during heat and moisture stress. Crop Sci., 5, 181.
- Maximov, N.A. (1929). The Plant in Relation to Water. Allen & Unwin, London.
- Maximov, N.A. (1931). The physiological significance of the xeromorphic structure of plants. J. Ecol., 19, 273-282.
- Mazur, P. (1966). Physical and chemical basis of injury in single-celled micro-organisms subjected to freezing and thawing. In Cryobiology (Meryman, H.T., ed.), 213-315. Academic Press, London and New York.
- Mazur, P. (1967). Physical-chemical basis of injury from intracellular freezing in yeast. In Cellular Injury and Resistance in Freezing Organisms, 171-189 (Asahina, E., ed.).
- Mazur, P. (1969). Freezing injury in plants. A. Rev. Pl. Physiol., 20, 419-448.
- Meador, E.N., Davidson, O.W. and Blake, M.A. (1945). A method for determining the relative cold hardiness of dormant peach fruit buds. J. agric. Res., 70, 283-302.

- Metcalfe, G. (1950). The ecology of the Cairngorms II. The Mountain Callunetum. J. Ecol., 38, 46-74.
- Michael, G. (1966). Untersuchungen über die winterliche Dürresistenz einiger immergrüner Gehölze im Hinblick auf eine Frost-trocknisgefahr. Flora, Jena, 156, 350-372.
- Michaelis, P. (1934). Ökologische studien an der alpinen Baumgrenze IV. Zur Kenntnis des winterlichen Wasserhaushaltes. Jb. wiss. Bot., 80, 169-247.
- Millener, L.H. (1962). Day-length as related to vegetative development in Ulex europaeus L. II. Ecotypic variation with latitude. New Phytol., 61, 119-127.
- Miller, E.C. (1917). Daily variations of water and dry matter in the leaves of corn and the sorghums. J. agric. Res., 10, 11, cited by Barrs (1968).
- Milthorpe, F.L. (1960). The income and loss of water in arid and semi-arid zones. Arid Zone Res., 15, 3-36.
- Modlibowska, I. and Rogers, W.S. (1955). Freezing of plant tissues under the microscope. J. exp. Bot., 6, 384-391.
- Monthly Weather Report. Meteorological Office, London.
- Moschkov, B.S. (1935). Photoperiodismus und Frosthärte ausdauernder Gewächse. Planta (Berl.), 23, 774-803.
- Newton, R. (1922). A comparative study of winter wheat varieties with especial reference to winter killing. J. agric. Sci., Camb., 12, 1-19.
- Olien, C.R. (1961). A method of studying stresses occurring in plant tissue during freezing. Crop Sci., 1, 26-28.

- Oliver, J. (1964). A study of upland temperatures and humidities in South Wales. Publs Inst. Br. Geogr., 35, 37-54.
- Oppenheimer, H.R. (1932). Zur Kenntnis der hochsommerlichen Wasserbilanz mediterraner Gehölze. Ber. dt. bot. Ges., 50A, 185-245.
- Oppenheimer, H.R. (1960). Adaptation to drought : xerophytism. Arid Zone Res., 15, 105-138.
- Oppenheimer, H.R. (1963). Zur Kenntnis kritischer Wasser-Sättigungsdefizite in Blättern und ihrer Bestimmung. Planta (Berl.), 60, 51-69.
- Oppenheimer, H.R. and Mendel, K. (1934). Some experiments on water relations of citrus trees. Hadar (Tel-Aviv), 7, 35-37, 59-61, 150-153.
- Osterhout, W.J.V. (1922). Injury, recovery and death in relation to conductivity and permeability. Lippincott, Philadelphia, Penn.
- Parker, J. (1953). Some applications and limitations of tetrazolium chloride. Science, 118, 77-79.
- Parker, J. (1956). Drought resistance in woody plants. Bot. Rev., 22, 241-289.
- Parker, J. (1962). Relationships among cold hardiness, water-soluble protein, anthocyanins, and free sugars in Hedera helix L. Pl. Physiol., Lancaster, 37, 809-813.
- Parker, J. (1963). Cold resistance in woody plants. Bot. Rev., 29, 123-201.
- Parker, J. (1968). Drought-resistance mechanisms. In Water Deficits and Plant Growth, Vol. I. (Kozlowski, T.T., ed.), 195-234, Academic Press, New York.

- Patten, D.T. (1963). Vegetational pattern in relation to environments in the Madison Range, Montana. Ecol. Monogr., 33, 375-406.
- Pears, N.V. (1968). The natural altitudinal limit of forest in the Scottish Grampians. Oikos, 19, 71-80.
- Peltier, G.L. (1931). Control equipment for the study of hardness in crop plants. J. agric. Res., 43, 177-182.
- Pharis, R.P. (1966). Comparative drought resistance of five conifers and foliage moisture content as a viability index. Ecology, 47, 211-221.
- Pharis, R.P. and Ferrell, W.K. (1966). Differences in drought resistance between coastal and inland sources of Douglas fir. Can. J. Bot., 44, 1651-1659.
- Pisek, A. (1950). Frosthärte und Zusammensetzung des Zellsaftes bei Rhododendron ferrugineum, Pinus cembra und Picea excelsa. Protoplasma, 39, 129-146.
- Pisek, A. (1956). Der Wasserhaushalt der Meso-und Hygrophyten. In Handbuch der Pflanzenphysiologie, Vol. 3., 825-853. (Ruhland, W. ed.), Springer-Verlag, Berlin.
- Pisek, A. (1958). Versuche zur Frostresistenzprüfung von Rinde, Winterknospen und Blüten einiger Arten von Obsthölzern. Gartenbauwissenschaft, 23, 54-74.
- Pisek, A. and Berger, E. (1938). Kutikuläre Transpiration und Trockenresistenz isolierter Blätter und Sprosse. Planta (Berl.), 28, 124-155.
- Pisek, A. and Cartellieri, E. (1933). Zur Kenntnis des Wasserhaushaltes der Pflanzen III. Alpine Zwergstraucher. Jb. wiss. Bot., 79, 131-190.

- Pisek, A. and Larcher, W. (1954). Zusammenhang zwischen Austrocknungsresistenz und Frosthärte bei Immergrünen. Protoplasma, 44, 30-46.
- Pisek, A. and Schiessl, R. (1947). Die Temperaturbeeinflussbarkeit der Frosthärte von Nadelhölzern und Zwergsträuchern an der alpinen Waldgrenze. Ber. naturw.-med. Ver. Innsbruck, 47, 33-52.
- Pisek, A. and Winkler, E. (1953). Die Schliesbewegung der Stomata bei ökologisch verschiedenen Pflanzentypen in Abhängigkeit. Planta (Berl.), 42, 253-278.
- Polunin, N. (1959). Circumpolar Arctic Flora. Clarendon Press, Oxford.
- Purcell, A.E. and Young, R.H. (1963). The use of tetrazolium in assessing freeze damage in citrus trees. Proc. Am. Soc. hort. Sci., 83, 352-358.
- Ritchie, J.C. (1955). Biological flora of the British Isles - Vaccinium vitis-idaea L. J. Ecol., 43, 701-708.
- Ritchie, J.C. (1956). Biological flora of the British Isles - Vaccinium myrtillus L. J. Ecol., 44, 291-299.
- Rouschal, E. (1938). Zur Ökologie der Macchien I. Der sommerliche Wasserhaushalt der Macchien-pflanzen. Jb. wiss. Bot., 87, 436-523.
- Ruhland, W. (ed). (1956). Handbuch der Pflanzenphysiologie, Vol. 3 - Water relations. Springer-Verlag, Berlin.
- Rychnovská, M. (1965). Water relations of some Steppe plants investigated by means of the reversibility of the water saturation deficit. In Water Stress in Plants (Slavik, B., ed.). 108-116.

- Rychnovská-Soudková, M. (1963). Study of the reversibility of the water saturation deficit as one of the methods of causal phytogeography. Biologia Pl., 5, 175-180.
- Rychnovská, M. and Květ, J. (1963). Water relations of some psammophytes with respect to their distribution. In The Water Relations of Plants (Rutter, A.J. and Whitehead, F.H. eds.) 190-198.
- Rychnovská, M. and Květ, J. (1965). Contribution to the ecology of the steppe vegetation of the Tihany peninsula. III. Estimation of drought resistance based on the saturation of water deficit. Annls. Inst. biol., Tihany, 32, 289-296.
- Sakai, A. and Otsuka, K. (1967). Survival of plant tissue at super-low temperatures. V. An electron microscope study of ice in cortical cells cooled rapidly. Pl. Physiol., Lancaster, 42, 1680-1694.
- Sakai, A. and Yoshida, S. (1967). Survival of plant tissue at super-low temperatures VI. Effects of cooling and rewarming rates on survival. Pl. Physiol., Lancaster, 42, 1695-1701.
- Salisbury, F.B., Spomer, G.G., Sobrol, M. and Ward, R.J. (1968). Analysis of an alpine environment. Bot. Gaz., 129, 16-32.
- Sampson, J. (1961). A method of replicating dry and moist surfaces for examination by light microscopy. Nature, Lond., 191, 932.
- Satoo, T. (1962). In Tree Growth (Kozlowski, T.T. ed.), Ronald Press, New York, cited by Barrs (1968).
- Schimper, A.F.W. (1898). Pflanzengeographie auf physiologischer Grundlage. Gustav Fischer, Jena.

Schimper, A.F.W. (1903). Plant-geography upon a physiological basis.

Authorized English Translation by Fisher, W.R. Clarendon Press, Oxford.

Schmutz, W., Sullivan, C.Y. and Levitt, J. (1961). Sulphydryls - a new factor in frost resistance II. Relation between sulphydryls and relative resistance of fifteen wheat varieties.

Pl. Physiol., Lancaster, 36, 617-620.

Schratz, E. (1932). Untersuchungen über die Beziehung zwischen Transpiration und Blattstruktur. Planta (Berl.), 16, 17-69.

Schroeter, C. (1926). Pflanzenleben der Alpen. Albert Raustein, Zurich.

Shantz, H.L. (1927). Drought resistance and soil moisture. Ecology, 8, 145-157.

Siminovitch, D. and Scarth, G.W. (1938). A study of the mechanism of frost injury to plants. Can. J. Res. C, 16, 467-481.

Slatyer, R.O. (1955). Studies of the water relations of crop plants grown under natural rainfall in northern Australia. Aust. J. agric. Res., 6, 365-377.

Slatyer, R.O. (1962). Internal water relations of higher plants. A. Rev. Pl. Physiol., 13, 351-378.

Slatyer, R.O. (1967). Plant-Water Relationships. Academic Press, New York.

Slatyer, R.O. and McIlroy, I.C. (1961). Practical Microclimatology.

CSIRO Australia for UNESCO, cited by Barrs (1968).

Slavik, B. (1966). Response of grasses and cereals to water. In The Growth of Cereals and Grasses (Milthorpe, F.L. and Ivins, J.D., eds.), 227-40. Butterworth, London and Washington D.C.

Smith, F.E. (1951). Tetrazolium salt. Science, 113, 751-754.

- Snedecor, G.W. and Cochran, W.G. (1967). Statistical Methods, 6th Edition.
Iowa State University Press.
- Sørensen, T. (1941). Temperature relations and phenology of the north-east Greenland flowering plants. Meddr. Grønland, 125, 1-305.
- Stålfelt, M.G. (1961). The effect of the water deficit on the stomatal movements in a carbon dioxide-free atmosphere. Physiologia Pl., 14, 826-843.
- Steponkus, P.L. and Lanphear, F.O. (1967). Refinement of the triphenyl tetrazolium chloride method of determining cold injury. Pl. Physiol., Lancaster, 42, 1423-1426
- Steponkus, P.L. and Lanphear, F.O. (1968). The role of light in cold acclimation of Hedera helix L. var. Thorndale. Pl. Physiol., Lancaster, 43, 151-156.
- Stocker, O. (1923). Die Transpiration und Wasserökologie nordwestdeutscher Heide - und Moorpflanzen am Standort. Z. Bot., 12, 1-41.
- Stocker, O. (1929). Das Wasserdefizit von Gefasspflanzen in verschiedenen Klimazonen. Planta (Berl.), 7, 382-387.
- Stocker, O. (1931). Transpiration und Wasserhaushalt in verschiedenen Klimazonen I. Untersuchungen an der arktischen Baumgrenze in Schwedisch-Lappland. Jb. wiss. Bot., 75, 494-549.
- Stocker, O. (1956). Die Dürre-resistenz. In Handbuch der Pflanzenphysiologie, Vol. 3. (Ruhland, W., ed.). 693-741.
- Stocker, O. (1956b). Die Abhängigkeit der Transpiration von den Umweltfaktoren. In Handbuch der Pflanzenphysiologie, Vol. 3. (Ruhland, W., ed.)., 436-488.

- Stocker, O. (1960). Physiological and morphological changes in plants due to water deficiency. Arid Zone Res., 15, 63-104.
- Stuart, N.W. (1939). Comparative cold hardiness of scion roots from fifty apple varieties. Proc. Am. Soc. hort. Sci., 37, 330-334.
- Till, O. (1956). Über die Frosthärte von Pflanzen sommer-grüner Laubwälder. Flora, Jena, 143, 498-542.
- Tranquillini, W. (1958). Die Frosthärte der Zirbe unter besonderer Berücksichtigung autochthoner und aus Forstgärten stammender Jungpflanzen. Forstwiss. ZentBl., 77, 89-105. cited by Tranquillini (1964).
- Tranquillini, W. (1963a). The dependence of carbon-dioxide assimilation by young larch, spruce and cembra pine trees on air humidity and soil moisture. Experiments in a climate-controlled wind tunnel. Planta (Berl.), 60, 70-94.
- Tranquillini, W. (1963b). Climate and water relations of plants in the sub-alpine region. In The Water Relations of Plants (Rutter, A.J. and Whitehead, F.H., eds.), 153-167.
- Tranquillini, W. (1964). The physiology of plants at high altitudes. A. Rev. Pl. Physiol., 15, 345-362.
- Tranquillini, W. (1967). Über die physiologischen Ursachen der Wald- und Baumgrenze. Mitt. forstl. BundVersAnst. Wien, 75, 457-87.
- Troll, C. (1956). Das Wasser als pflanzengeographischer Faktor. In Handbuch der Pflanzenphysiologie, Vol. 3 (Ruhland, W., ed.), 750-786.

- Tumanov, I.I. (1927). Ungenügende Wasserversorgung und das Welken der Pflanzen als Mittel zur Erhöhung ihrer Dürre-resistenz. Planta (Berl.), 3, 391-480.
- Tumanov, I.I. (1967). The frost-hardening process of plants. In The Cell and Environmental Temperature (Troshin, A.S., ed.) 6-14.
- Ulmer, W. (1937). Über den Jahresgang der Frosthärte einiger immergrüner Arten der alpinen Stufe, sowie der Zirbe und Fichte. Jb. wiss. Bot., 84, 553-592.
- Vaadia, Y., Rancy, F.C. and Hagan, R.M. (1961). Plant water deficits and physiological processes. A. Rev. Pl. Physiol., 12, 265-292.
- Vaartaja, O. (1959). Evidence of photoperiodic ecotypes in trees. Ecol. Monogr., 29, 91-111.
- van der Veen, R. and Meijer, G. (1959). Light and Plant Growth. Philips' Technical Library.
- Walter, H. (1955). The water economy and the hydrature of plants. A. Rev. Pl. Physiol., 6, 239-252.
- Walter, H. (1963). Zur Klärung des spezifischen Wasserzustandes im Plasma und in der Zellwand bei der höheren Pflanze und seine Bestimmung II. Methodisches. Ber. dt. bot. Ges., 76, 54-71.
- Warming, E. (1895). Plantesamfund. Grundtræk af den økologiske Plantegeografi. Kjöbenhavn.
- Warming, E. (1909). Oecology of Plants. (Translation by Groom, P. and Balfour, I.B.) Clarendon Press, Oxford.
- Watson, E.V. (1968). British Mosses and Liverworts, 2nd Edition. Cambridge University Press.

- Weatherley, P.E. (1950). Studies in the water relations of the cotton plant. I. The field measurement of water deficits in leaves. New Phytol., 49, 81-97.
- Weatherley, P.E. (1965). The state and movement of water in the leaf. Symp. Soc. exp. Biol., 19, 157-184.
- Wilner, J. (1955). Results of laboratory tests for winter hardiness of woody plants by electrolytic methods. Proc. Am. Soc. hort. Sci., 66, 93-99.
- Wilner, J. (1959). Note on an electrolytic procedure for differentiating between frost injury of roots and shoots in woody plants. Can. J. Pl. Sci., 39, 512-513.
- Wilner, J. (1960). Relative and absolute electrolytic conductance tests for frost hardiness of apple varieties. Can. J. Pl. Sci., 40, 630-637.
- Wilner, J. (1961). Relation between certain methods and procedures of testing for winter injury of outdoor exposed shoots and roots of apple trees. Can. J. Pl. Sci., 41, 309-315.
- Wilner, J., Kalbfleisch, W. and Mason, W.J. (1960). Note on two electrolytic methods for determining frost hardiness of fruit trees. Can. J. Pl. Sci., 40, 563-565.
- Winkler, A. (1913). Über den Einfluss der Aussenbedingungen auf die Kälteresistenz ausdauernder Gewächse. Jb. wiss. Bot., 52, 467-506.
- Worzella, W.W. and Cutler, G.H. (1941). Factors affecting cold resistance in winter wheat. J. Am. Soc. Agron., 33, 221-230.
- Zobel, D.B. (1969). Factors affecting the distribution of Pinus pungens, an Appalachian endemic. Ecol. Monogr., 39, 303-33.